ANDROGENS

AND

ANDROGEN RECEPTORS

IN

PROSTATIC CANCER

ANDROGENS AND ANDROGEN RECEPTORS IN PROSTATIC CANCER

ANDROGENEN EN ANDROGEENRECEPTOREN BIJ PROSTAATCARCINOOM

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR

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PROF. DR. A.H.G. RINNOOY KAN

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PROMOTOR : Prof.Dr. F.H. Schröder
OVERIGE LEDEN: Prof.Dr. S.W.J. Lamberts
Prof.Dr. H.J. van der Molen
Prof.Dr. H.J. de Voogt
COPROMOTOR : Dr. M.A. Blankenstein

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Opgedragen aan de patiënten die
dit onderzoek mogelijk maakten

CONTENTS

CHAPTER 1

SCOPE OF THIS THESIS

1.1 Endocrine dependency of prostatic cancer

Our understanding of the testicular control of growth and functioning of the accessory sex glands began with an observation in the 18th century of John Hunter (1), who discovered in animals the endocrine dependency of the prostate. He demonstrated that castration in experimental animals causes a decrease in the volume of the prostate. White (2) reported in 1895 favourable results of "double" castration in men with hypertrophy of the prostate and one year later Cabot (3) proposed castration as treatment for an enlarged prostate in general. Differentiation between carcinoma and BPH was not done.

The studies of Huggins and Hodges (4,5) in the early 1940's defined the regulatory role of the testes and testicular androgens in prostatic cancer. Their work placed the orchiectomy for treatment of prostatic cancer on a scientific basis and brought its general acceptation. Since then different forms of hormonal therapy have been used in all stages of prostatic cancer. Today, in the 80's, endocrine manipulation is generally accepted as first treatment in disseminated disease, although the impact of hormonal manipulation on patient survival (6,7) and the optimal timing of the initation of treatment are still controversial (8-

10). After more than 45 years of investigation no single hormonal treatment has proven to be superior, so the conclusion of Scott in an overview (11) on hormonal therapy for prostatic cancer: "We have gone as far as we can go in the hormonal treatment of advanced prostatic cancer, and it is unlikely that further search will reveal a better treatment than castration - estrogen therapy", may still be valid. This statement, although seemingly definite, bears the challenge to develop modalities of treatment which are more effective than endocrine manipulations. As a part of future treatment it will be necessary to find methods that enable us to predict which patient will and which patient will not have a prolonged response to hormonal treatment. The estimation of nuclear androgen receptor (ARn) seemed promissing according to the findings of Trachtenberg and Walsh (12) and Ghanadian et al (13). The present thesis is aimed to contribute to this problem.

1.2 Objectives of this study

It is generally accepted that steroid hormones achieve at least part of their effects on target cells through binding to intracellular receptor proteins (14). As a consequence the sensitivity of human breast cancer to endocrine measures has been related to the presence and concentration of the estrogen receptor protein. It is now evident that the selection of treatment for patients with advanced breast cancer can be greatly improved by quantitation of estrogen and progestin receptors (15). By analogy with the situation in

breast cancer, the hormonal responsiveness of prostatic cancer was considered to be related to the presence of androgen receptors. In breast cancer receptors are measured in a cytosol of the tissue. In prostate cancer, the predominant nuclear localization (16) of the androgen receptor precludes the use of cytosol. Therefore, in prostatic tissue, androgen receptors should be measured in a nuclear preparation.

Ghanadian et al (13) and Trachtenberg and Walsh (12) were the first who reported on a relationship between the nuclear androgen receptor (ARn) content of prostatic tissue and the duration of response on hormonal treatment in patients with metastatic disease of the prostate. Although most prostatic cancers initially are androgen dependent (17) the objective response to therapeutic measures that lower circulating androgens (6) is of only limited duration in approximately 30 percent of patients. If an androgen receptor assay could identify these poorly responding patients, non endocrine therapy could be started earlier, i.e. at a time when they are more likely to tolerate the side effects of such treatments.

When prostatic cancer is diagnosed, the disease has often progressed to such an extent already that prostatic surgery is not considered and systemic therapy is given. The amount of tissue which becomes available for receptor estimation is, therefore, limited to a few needle biopsies. An assay which would allow measurement of androgen receptors in such small samples could also be used to compare androgen recep-

tor levels in histologically normal, hyperplastic and malignant prostatic tissue in an attempt to evaluate whether hyperplasia or prostatic cancer are associated with changes in the androgen receptor content and to monitor the changes in the tumor cell populations present in the primary tumor and in the metastases during treatment.

The objectives of the study described in this thesis, therefore, were:

a. to study the nuclear androgen receptor level in biopsy specimens of normal, hyperplastic, and malignant human prostatic tissue.
b. to evaluate the prognostic significance of nuclear androgen receptor levels in patients with advanced prostate cancer who are treated by orchiectomy.

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CHAPTER 2

INTRODUCTION

2.1 Rationale for hormonal therapy

2.1.1 Hormonal influences on the prostate

Prostatic cells are not able to grow or function in the absence of androgens (1,2). The aim of hormonal treatment is to deprive the prostatic tumor cells of androgens and their byproducts. The most potent androgen is dihydrotestosterone which is produced in the prostatic cells from testosterone. Ninety percent of circulating testosterone is produced by the testes. The adrenal androgens, dehydroepiandrosterone, dehydroepiandrosterone sulfate and androstenedione are weak androgens and by themselves they are not capable of maintaining prostatic growth and function (3). Testosterone, the principal hormone of the testis, is synthesized from cholesterol in the Leydig cells. About 97 percent of testosterone in plasma is bound to proteins like sex hormone-binding globuline (SHBG) and albumin and only three percent is unbound. Only the free testosterone is considered to be functionally active. The androgen biosynthesis of the Leydig cells and the adrenal glands, is regulated by the pituitary through two separate feedback mechanisms respectively. The positive mediators are luteinizing hormone (LH) and adrenocorticotropic hormone (ACTH), the negative mediators are testosterone and cortisol. The pituitary in turn is

controled by the median eminence of the hypothalamus through the mediators luteinizing hormone-releasing hormone (LHRH) and corticotropin-releasing factor (CRF). Prolactin is another pituitary hormone which influences androgen metabolism in the prostate (4). Its release is inhibited by prolactin inhibiting factors (PIF's), the most important of which is dopamine, while there are also positive mediators originating from the hypothalamus. The effect of prolactin on the prostate is dependent on the presence of androgen, their actions are synergistic (5,6). The role of estrogens in the interaction of the hypothalamus, pituitary, testis and adrenals and prostate consists mainly of an inhibition of LH secretion and PIF. Estrogens also induce an increase in SHBG so free testosterone is reduced, and they might have a direct effect on prostatic cells.

2.1.2 The Androgen Receptor

Unbound testosterone diffuses passively through the prostatic cell membrane into the cytoplasm, where it is reduced to dihydrotestosterone (DHT) by the enzyme 5α-reductase. DHT then binds to the androgen receptor. Originally a cytoplasmic DHT receptor complex was thought to be formed which was to be translocated to the nucleus (7). Recent evidence, however, suggests that steroid receptors are permanently localized in the nuclear compartment (8-11). For estrogen and progestin receptors growing evidence with respect to nuclear localization is obtained (9,10) the glucocorticoid receptor however has been found in both cytoplasm and

nucleus (11). For the androgen receptor unfortunately no clear cut conclusions can be drawn yet due to the lack of monoclonal antibodies. The androgen receptor has been cloned and sequenced (12-14). Therefore, this information will probably become available shortly. By analogy with the estrogen and progestin receptor and supported by the presence of 5α-reductase in the nuclear membrane (15) and the morphologic localization of androgen receptors (16) it is tempting to speculate that the androgen receptor also resides permanently in the nucleus. A scheme of the possible mechanism of action of androgens is given in Figure 2.1. Regardless of

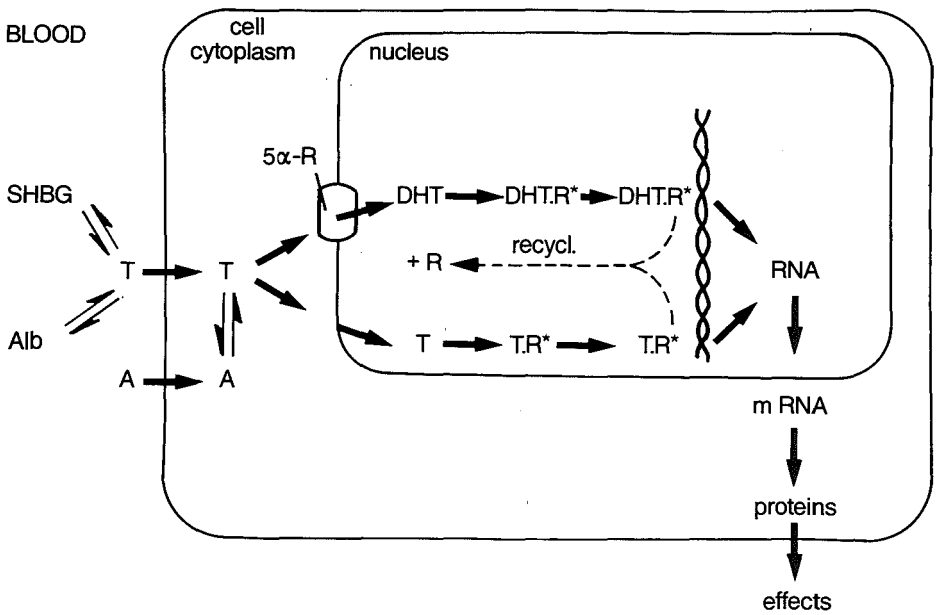

Figure 2.1. Scheme of possible mechanism of action of androgens in the prostatic cell. Abbreviations used: SHBG - Sex Hormone Binding Globulin; Alb - Albumin; T - testosterone; A - Androstenedione; 5α-R - 5α-Reductase; DHT - Dihydrotestosterone; R - Receptor; R* - activated receptor.

the localization of the unoccupied receptor, broad consensus exists on association of the DHT-receptor complex with chromatin, activating DNA to produce messenger RNA which in turn starts protein synthesis, essential for metabolic functions of the prostatic acinar cell. Recently Mobbs et al (17,18) and van Steenbrugge et al (19) postulated a role for estrogens in the regulation of the androgen receptor.

2.2 Different forms of hormonal treatment

The principal goal of hormonal therapy in the treatment of prostatic cancer is the suppression of androgenic stimuli of the prostate. Along the pathway between androgen production and their ultimate effect on the prostatic cancer cell there are many points at which the cycle may be interrupted. Several possibilities then exist for deprivation of the tumor cells of androgenic stimuli e.g.: bilateral orchiectomy, estrogens, hypophysectomy, adrenalectomy, anti-androgens, luteinizing hormone releasing hormone analogues and total androgen suppression. Combination of these principles may lead to simultaneous suppression of testicular and adrenal androgens (total androgen withdrawal). A schematic view of the possiblities for therapeutic intervention of prostatic cancer is given in Figure 2.2.

2.2.1 Bilateral Orchiectomy

Orchiectomy, or subcapsular orchiectomy causes prompt decline of plasma testosterone levels. It is the standard form

of endocrine therapy since the study by Huggins and Hodges (21). Androgens derived from the adrenals are not affected. Whether the androgens of adrenal origin play a signifcant

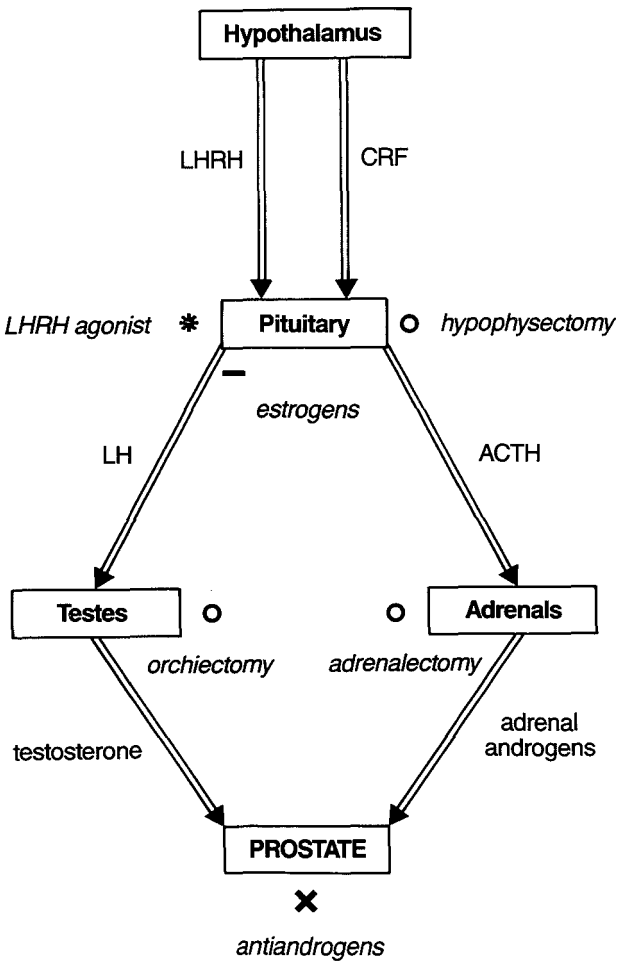

Figure 2.2. Diagram of hormonal stimulation of prostate growth and possibilities for therapeutic intervention. Symbols: X blockade; - negative feedback; * desensitization; o surgical ablation.

role in prostatic cancer is highly unlikely. Oesterling and Walsh (3) in a autopsy study of men with hypogonadotropic hypogonadism and panhypopituitarism demonstrated the inability of adrenal androgens to stimulate the adult human prostate. Orchiectomy is a simple and safe operation, which can be done under local anesthesia on an out-patient basis. Vasomotor hot flushes, which can be very distressing for the patients may occur, but they can be suppressed with a low dose of estrogens or an anti-androgen. The major drawback of orchiectomy is the psychological impact of castration, with a thorough explication including advantages and disadvantages of alternative forms of treatment and avoiding the word "castration", most patients will choose for orchiectomy. Currently, there is no convincing evidence that any form of endocrine therapy, including the methods of total androgen withdrawal is more effective than orchiectomy.

2.2.2 Estrogen Administration

In the intact male estrogens act primarily by suppressing release of LH from the pituitary and thus decrease endogenous production of testosterone. Marked suppression of serum testosterone is achieved rapidly and maintained indefinitely if the application of proper estrogenic hormones in appropriate dosage is continuous. In addition, estrogens increase the plasma level of sex hormone binding globulin, thereby reducing the percentage of unbound testosterone. The side effects of oral estrogens are known and have been well documented (21). The major complication is the increased

incidence of deep venous trombosis and cardiovascular deaths with the higher dosages 3 and 5 mg (22,23). These complications are less severe with a dose of 1 mg per day. This dose, does not suppress plasma testosterone to castration level. Because of these major complications estrogens are no longer a first choice of treatment.

2.2.3 Hypophysectomy

The role of pituitary ablation in an attempt to lower extratesticular androgens is doubtful because adrenal androgens have no stimulating effect on prostatic growth in the absence of testes in the adult human prostate (3). This therapy has only been used in patients who fail to respond or who suffer relapse after conventional hormonal therapy for prostatic cancer, and has been associated with objective responses of short duration and subjective improvement, mainly pain relief lasting 3-6 months on average (24).

2.2.4 Adrenalectomy

The adrenal androgens are weak androgens and they have no stimulating effect on the human prostate and they are not capable of supporting prostatic growth (3). In a review (27) Brendler reported a 73 percent subjective improvement rate after adrenalectomy, but only a 6 percent objective response rate, the duration of response is mostly short. Adrenalectomy can be achieved surgically or chemically by aminoglutethimide and hydrocortisone. In view of the impact of the

operation and the many side effects of chemical adrenalectomy and in face of the doubtful clinical responsiveness of advanced pretreated prostatic cancer this form of treatment is no longer frequently used.

2.2.5 Antiandrogens

These agents exert their effect by direct competition with androgens at target organs. By competing with androgens for receptor sites anti-androgens inhibit the formation and or nuclear translocation or tight nuclear association in the new concept of the receptor-dihydrotestosterone complex (25). Two types of antiandrogens can be distinguished, i.e. the pure antiandrogens and the antiandrogens of the cyproterone acetate type (26).

By blocking the negative feed-back mechanism of androgens in the hypothalamus and pituitary and consequently rise of testosterone after protracted treatment the pure antiandrogens should be combined with a LHRH agonist or an inhibitor of gonadotropin secretion. Antiandrogens of the cyproterone-acetate type inhibit gonadotropin secretion and androgen biosynthesis, they block testicular and extratesticular androgens within the prostate. In numerous clinical trials response rates appear to be similar to those obtained with orchiectomy or estrogens (27,28).

2.2.6 Luteinizing hormone - releasing hormone analogues

After Schally and associates elucidated the structure of naturally occuring luteinizing hormone - releasing hormone

(29) many synthetic analogues have been developed. These analogues have considerably longer half lives and are therefore much more potent than native LHRH. The effect of LH-RH agonists is biphasic. In the inital phase the LH-RH agonist stimulates pituitary LH secretion and consequently androgen biosynthesis. Following this initial phase which lasts for about 2 weeks, the pituitary LH-RH receptor is down regulated. As a result the pituitary becomes refractory or insensitive to further stimulation by LH-RH secretion and testosterone biosynthesis decreases to castration levels. Because of the transient rise in plasma testosterone, patients may experience a flare-up of the disease, prior to the response. LHRH can be administered intravenously (29), or intranasally (30). Recently, depot formulations have been designed which are equally effective (31) to avoid repeated injections or inhalations.

2.2.7 Total androgen suppression

By a combination of the above mentioned methods or by some new drugs e.g. potent aromatase inhibitors (32) it is possible to suppress the effects of testicular and adrenal androgens. In the past this form of treatment has been used as a second line endocrine management for patients who relpased after castration or estrogen therapy. Ablation of adrenal androgens with adrenalectomy, adrenal suppression by corticosteroids or by hypophysectomy as second line of treatment produces low objective response rates (24). The availability

of new drugs as LHRH analogs, which can achieve castration levels of serum testosterone, gave rise to a revisited discussion about the aforementioned issue. Labrie et al (33) claimed a remarkable increase in efficacy in the treatment of patients with M+ disease by LHRH analogs combined with an anti-androgen as primary treatment over castration alone. The result of this controled, nor randomized nor prospective study with an average follow-up of only 4.2 months (1-12) must be considered with great caution. The question if one can increase the survival of a patient treated early with LHRH and an anti-androgen beyond that observed with castration alone can only be answered by controled randomized prospective studies. Untill these data become available, total androgen suppression, will remain controversial as primary means of management (34,35).

2.3 The timing of androgen ablative therapy

Soon after its introduction hormonal therapy was used for almost every stage of prostatic carcinoma (36). Today hormonal therapy is accepted unanimously for patients with symptomatic stage D2 disease. Since several studies of the Veteran Administration Cooperative Urological Research Group (VACURG) (37,38) and a study by Lepor et al (39) suggested that endocrine therapy is only palliative and does not prolong life. Considerable controversy exists as to when to start this treatment in patients with D1 stage disease (40). Since endocrine therapy is only a palliative measure it should according to some investigators (40,41) be withheld

until the patient becomes symptomatic. Others (42,43) believe that patients with systemic disease have to be treated by endocrine means as soon as possible. They advocate early treatment considering the possible advantages of early hormonal treatment in patients who are responders to be:
1. delay of progression, 2. prolongation of the symptom free interval, 3. preservation of quality of life, 4. earlier recognition of the patients who do not respond and who may in the future be treated with chemotherapy at a moment when they are in a better clinical condition, and the tumor burden is smaller. In view of the aforementioned controversy we studied the "early" orchiectomy in patients with D1 prostatic carcinoma carefully staged by pelvic lymphadenectomy. In this study an attempt was made to evaluate the effect of early hormonal treatment in patients with only nodal disease. The results of this study are presented in Appendix I and show that time to treatment failure in this group compared favourably to progression of patients treated with other treatment modalities as reported in literature. Therefore, we advocate early treatment of patients with stage D1 disease. Only a randomised study in patients with D1 disease proven by pelvic lymphadenectomy, however, can give a definite answer to the question whether early hormonal therapy may provide an advantage in terms of a prolonged survival.

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CHAPTER 3

RECRUITMENT OF PATIENTS, COLLECTION AND PROCESSING OF SPECIMENS

3.1 Patients

In 1981 this prospective study on the aims as described in Chapter I was started. In the period 1981 - 1984, 115 new patients referred under the suspicion of prostatic carcinoma to the urological out patient clinics of four hospitals in Rotterdam (Academisch Ziekenhuis Rotterdam, St. Franciscus Ziekenhuis, Zuiderziekenhuis, Bergweg Ziekenhuis), gave their informed consent to cooperate in this study. During the same time a similar group of patients who underwent a TUR for benign prostatic hyperplasia was added as a control group. A 10 cc heparinized blood sample was taken for testosterone and SHBG measurement prior to the perineal biopsy or, as in the BPH group, before TUR. With the patient in lithotomy position, and after infiltration of the perineum with 10-20 cc 1% lidocaine, 2-4 perineal biopsies were taken in addition to the biopsies required for diagnosis. The tissue was immediately frozen in liquid nitrogen and stored at -80 C.

3.2 Staging of the Patients

Once prostatic cancer was diagnosed, the patient was carefully staged according to the TNM system. This includes

physical examination, a chest X-ray, a bone scan and a specific laboratory analysis. Only the group of 42 patients with M1 disease who were treated by bilateral orchiectomy and who had no hormonal treatment before, were followed in order to evaluate the prognostic significance of the nuclear androgen receptor level of their tumor.

3.3 Follow-up of the patients and definition of progression

Follow up consisted of physical examination and determination of serum alkaline and prostatic acid phosphatase levels every 3 months. Bone scan and chest X-ray were performed at 6 - 12 months intervals. In this study, time to progression was used to evaluate the duration of hormonal therapy in these patients.

Progression of disease was defined as - appearence of new distant metastases on chest ray or bone scan, - elevation of previously normal prostatic acid phosphatase level at 2 consecutive follow up visits, - appearance of biopsy proven soft tissue metastases, or - increase in volume of the primary tumor proven by ultrasound, or digital examination.

3.4 Tissues

Cryostat sections of 3μ thickness were cut lengthwise from each single frozen biopsy. Care was taken not to thaw the samples at this stage, the remainder of the biopsy was restored at -80 C. The frozen section was stained with haematoxylin - eosin for histological diagnosis. When the

histological analysis showed severe signs of infection or when less than 50% cancer cells were present in the whole biopsy, the biopsy was excluded from this study.

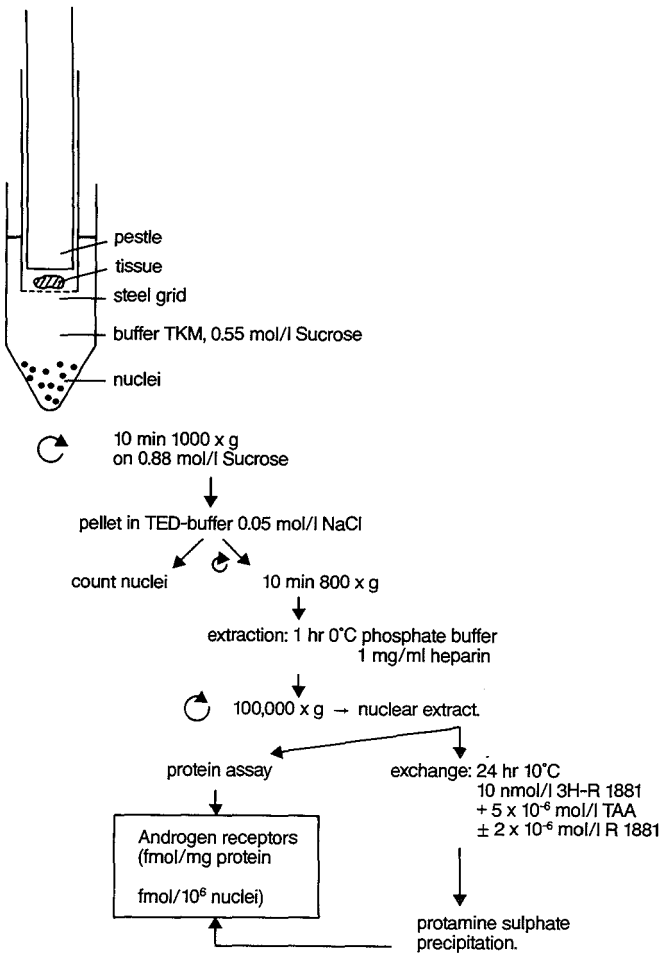

Figure 3.1. Diagrammatic outline of the assay for nuclear androgen receptors in prostatic biopsies.

3.5 Assay of nuclear androgen receptors

For the receptor estimation a minimal amount 25 mg of tissue was needed. When more than 50 mg were available more than one receptor assay was done. A detailed description of the receptor assay is given in Chapter 4, a diagrammatic outline of the assay for nuclear androgen receptors in prostatic biopsies is given in Figure 3.1. Briefly, nuclei were squeezed out of the tissue, and partly purified by centrifugation through 0.88 M sucrose. Receptors were extracted with heparin and quantified after exchange labeling with 3H-R1881 and protamine sulphate precipitation.

3.6 Statistical analysis

Nonparametric tests, e.g. the Spearman's rank correlation and the Wilcoxon test for unpaired samples were used for statistical analysis of the results. P-values smaller than 0.05 were considered to reflect statistical significance.

CHAPTER 4

NUCLEAR ANDROGEN RECEPTOR CONTENT IN BIOPSY SPECIMENS FROM HISTOLOGICALLY NORMAL, HYPERPLASTIC, AND CANCEROUS HUMAN PROSTATIC TISSUE

OGJM van Aubel, J Bolt-de Vries, MA Blankenstein, FJW ten Kate and FH Schröder.

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The Prostate 6:185–194 (1985)

Nuclear Androgen Receptor Content in Biopsy Specimens From Histologically Normal, Hyperplastic, and Cancerous Human Prostatic Tissue

O.G.J.M. van Aubel, J. Bolt-de Vries, M.A. Blankenstein, F.J.W. ten Kate, and F.H. Schröder

Departments of Urology (O.G.J.M.v.A., J.B.d.V., M.A.B., F.H.S.) and Pathology (F.J.W.t.K.), Medical Faculty, Erasmus University, Rotterdam, The Netherlands

Androgen receptors (ARn) were assayed in nuclear extracts of prostatic biopsies from 60 patients with benign prostatic hyperplasia (BPH) and 82 patients with prostatic cancer (PC), with an exchange assay using heparin extraction, labelling with ^{3}H-R1881, and protamine sulphate precipitation. The content of ARn of BPH biopsies (38 ± 34 fmol/mg protein [mean ± SD]; n = 70) was not different from that of PC biopsies (39 ± 32 fmol/mg protein; n = 115). Biopsies showing essentially normal prostatic tissue had a lower ARn content (12 ± 13 fmol/mg protein; n = 6). The content of ARn was independent of the age of the patient and of the histological grade of the carcinomas. A considerable variation in ARn content within tumors of individual patients was found, indicating that ARn are not uniformly distributed over prostatic tissue; ie, cells with high and low receptor content may coexist in different proportions in different regions of the prostate. Therefore, assays on multiple biopsies may be required for a proper estimation of the mean receptor content. The question remains, however, whether the behavior of the tumor is adequately predicted by the mean receptor level or, for instance, by the region with the lowest receptor content.

Key words: human prostate, nuclear androgen receptors (ARn), BPH, PC, normal, biopsies

INTRODUCTION

Patients with advanced prostatic cancer are likely to be subjected to hormonal therapy in order to suppress androgenic stimulation of malignant prostatic growth. Although most patients will demonstrate an initial response to endocrine treatment [1,2], the duration of response as well as the survival are variable [3]. Until now it has not been possible to select patients who will benefit from hormonal treatment for only a relatively short time. If the duration of the hormone sensitivity of the tumor could be predicted, however, patients could be spared the side effects of hormonal therapy and be treated earlier with alternative forms of treatment. By analogy with breast cancer, the androgen receptor content of prostatic tumors has been investigated as a means of predicting the response to endocrine therapy. The presence of androgen receptors (ARn) was shown in normal and pathologic prostatic tissue [4–11]. As a result of the different assays used and the small groups of patients studied, the clinical value of the the estimation of ARn is still in doubt. There are indications, however,

M.A. Blankenstein's present address is Department of Biochemistry, Rotterdam Radio-Therapeutic Institute, Rotterdam, The Netherlands.

Address reprint requests to O.G.J.M. van Aubel, Department of Urology, Erasmus University, Postbox 1738, 3000 DR Rotterdam, The Netherlands.

that estimation of ARn in prostatic tissue may be of importance in the future in predicting the effect of hormonal therapy. Ekman et al [12], Ghanadian et al [13], and Trachtenberg and Walsh [14] showed a relation between the quantity of cytoplasmic and nuclear androgen receptors and the duration of response to endocrine measures. Trachtenberg and Walsh [14] and Ghanadian et al [13], both estimating cytoplasmic as well as nuclear androgen receptors, found that only nuclear androgen receptor content did correlate with duration of response [13,14] and survival [14] following hormonal therapy in advanced prostatic cancer.

Since patients with advanced prostatic carcinoma are not eligible for open surgical procedures, biopsy of the prostate is the only acceptable alternative to provide us with small amounts of tissue (~ 25 mg per biopsy).

A method for the estimation of nuclear androgen receptors has been published [15], and recently this method has been made applicable to biopsy specimens [18]. Using relatively large samples (~ 200 mg) differences have been shown in the nuclear androgen receptor content of normal and pathologic human prostatic tissue [19,20]. The aim of the present investigation was to find out whether these reported differences could be confirmed using biopsies as small as 25 mg and to start a prospective study on the clinical value of this nuclear estimation in biopsy specimens of prostatic carcinoma.

MATERIALS AND METHODS

Tissue

Prostatic tissue was obtained from two different groups of patients by means of two to four perineal prostatic biopsies. One group of patients n = 83 (aged 47–89) underwent a diagnostic biopsy of their prostates. The other group of patients n = 60 (aged 57–91) had a biopsy of the prostate before undergoing TUR of their prostates for benign prostatic hyperplasia. The tissue was placed in liquid nitrogen immediately and stored at −80°C. Prior to the receptor assay, a frozen section was made of each single biopsy for histological diagnosis. If the amount of tissue exceeded 50 mg, more than one receptor assay was done. Histologic analyses of these biopsies showed that 10 out of 322 were normal, 140 showed BPH, 172 showed carcinomas. Tissue showing severe signs of infection was excluded from this study, as was tissue showing less than 50% cancer cells. In the BPH group a rough estimate was made of the percentage of epithelium in the specimen. Thus four groups were formed: group I (no epithelium), group II (< 50% epithelium), group III (~ 50% epithelium), group IV (> 50% epithelium).

Assay of Androgen Receptors

Androgen receptor assay was done essentially as described before [15,18]. After taking a frozen section for histological diagnosis, the remainder of the tissue (25–50 mg) was placed in a miniaturized steel agrid device consisting of a 10-cm-long stainless steel tube (8 mm in diameter) with a bottom of stainless steel gauze (80 mesh) and a stainless steel pestle covered with the same gauze. The tissue was squeezed between the two layers of gauze while the entire device was immersed in 2 ml ice-cold 0.50 mmol/liter Tris HCl buffer, pH 7.5, containing 2.5 mmol/liter KCl, 5mmol/liter $MgCl_2$ TKM buffer, and 0.55 mol/liter sucrose. The nuclei were concentrated and partially purified by layering the suspension over TKM buffer containing

0.88 mol/liter sucrose and centrifugation for 10 min at 1,000g. The nuclear pellet was resuspended in 10 mmol/liter Tris HCl-buffer, pH 7.5, containing 1.5 mmol/liter EDTA, 1.5 mmol/liter dithiothreitol (TED-buffer), and 50 mmol/liter NaCl. For extraction of androgen receptor complexes, the nuclear pellet was suspended in 0.1 ml 2 mmol/liter phosphate buffer, pH 8.5, containing 1 gm/liter heparin.

After 1 hr, the suspension was centrifuged for 30 min at 100,000g, and the supernatant was termed the nuclear extract. The nuclear extracts were incubated in siliconized glass tubes at 10°C in the dark, in the presence of 10^{-8} mol/liter ^{3}H-R1881 (methyltrienolone, specific activity 87 Ci/mmol; New England Nuclear; Dreieich, Federal Republic of Germany). Triamcinolone acetonide (5×10^{-6} mol/liter) was added to block the binding of ^{3}H-R1881 to progestin receptors possibly present in the nuclear extract. Correction for aspecific binding was made by a parallel incubation in the presence of a 200-fold excess unlabelled R-1881. The incubation volume was 40 μl. The protamine sulphate precipitation assay was performed on 25 μl of the labelled extracts as described by Foekens et al [15], and the precipitates were counted for 20 min in a Searle type Isocap-300 liquid scintillation counter. As reported earlier [15], extraction of androgen receptors from human prostate nuclei with heparin is twice as efficient as extraction with 0.4 M KCl.

Other Procedures

The protein concentration of nuclear extracts was determined according to Peterson [16]. DNA was estimated in nuclear pellets with the method of Hinegardner [17].

Statistical Evaluation

The significance of differences was tested by Wilcoxon's test. Differences were considered to be statistcially significant when p values less than 0.05 were obtained. The existence of correlations was tested with Spearman's rank correlation test.

RESULTS

Androgen receptors (ARn) were detected in the nuclear extracts of 103 out of 115 samples (90%) from prostatic carcinoma, in 66 out of 70 samples (94%) from hyperplastic prostates, and in four out of six samples from tissue that was histologically classified normal but obtained from benign hyperplastic or malignant prostates. The results of the measurement of androgen receptors in the samples are summarized in Table I. A large variation was observed in the results obtained for each type of tissue. Nevertheless a statistically significant difference was observed between the ARn content of normal tissue and hyperplastic or carcinomatous tissue ($p < 0.01$). No difference was observed between the ARn content of samples from hyperplastic and carcinomatous tissue. For prostatic carcinoma, no relation was found between the ARn content and the histological grade of the tumor (Table I). For hyperplastic prostates, a significant correlation ($R_s = 0.3586$; $p < 0.001$) was found between the estimated percentage of epithelium and the ARn level (Table I; Fig. 1). The nuclear androgen receptor levels of the biopsies of BPH and PC were independent of the age of the patients (Fig. 2).

More than one receptor estimation was performed in 31 patients. The actual receptor levels found for these patients are shown in Figure 3. A considerable

TABLE I. Nuclear Androgen Receptors (AR_n) in Human Prostatic Biopsies

Diagnosis	Estimated % epithelium (BPH) or histological grade (PC)	No. of samples	AR_n (fmol/mg nuclear extract protein) Mean	SD	Range
Normal prostate		6	12	13	0- 29
Hyperplasia	All	70	38*	34	0-189
	>50	17	56**	51	5-189
	~50	32	37**	26	0-118
	<50	13	31	22	0- 70
	0	8	18	22	5- 18
Carcinoma	All	115	39*	32	0-154
	G1	6	36	33	0- 86
	G2	40	30	23	0- 88
	G3	69	44	35	0-154

*$p < 0.01$ vs normal prostate (Wilcoxon's test).
**$p < 0.01$ vs normal hyperplasia containing no epithelium (Wilcoxon's test).

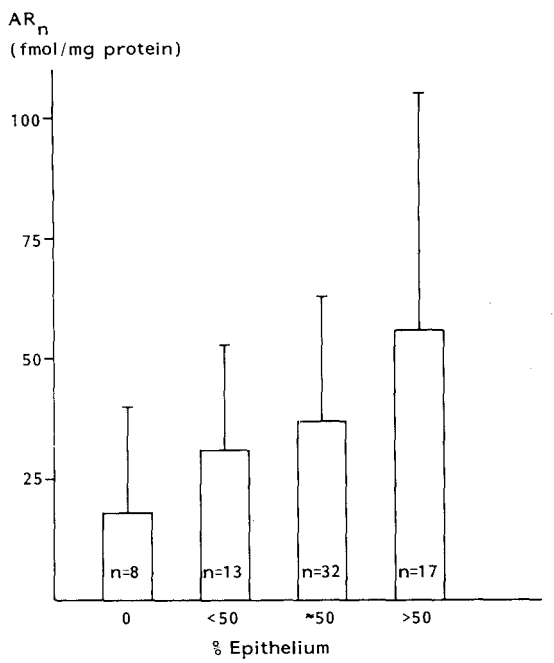

Fig. 1. Androgen receptor content of nuclear extracts (ARn) of biopsies of benign hyperplastic prostatic tissue (BPH) as a function of the estimated percentage of epithelium in the specimen. Results are expressed as means ± SEM.

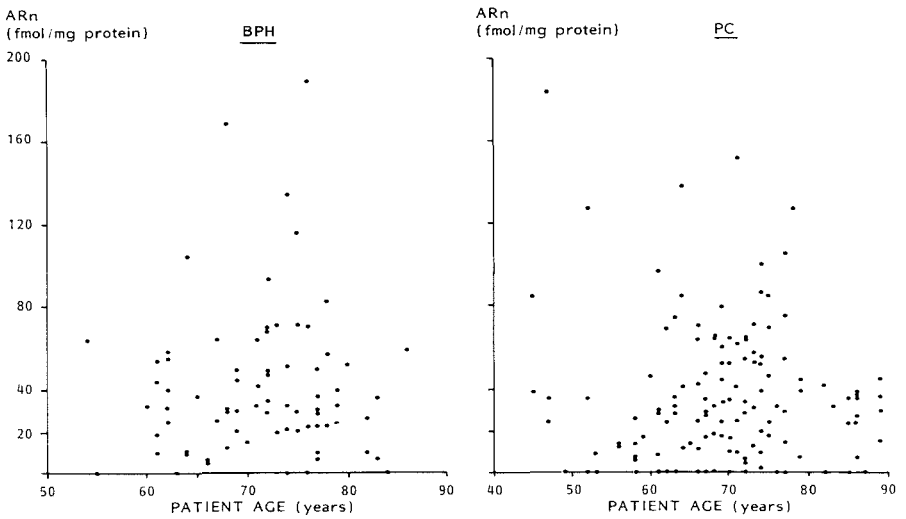

Fig. 2. Nuclear androgen receptor content (ARn) of prostatic biopsies histologically classified as benign hyperplasia (BPH) or carcinoma (PC) as a function of the age of the patients. No correlation was observed ($R_s = -0.001$ and 0.065, respectively).

variation in receptor content was found within the biopsies of the same tumors of these patients. For other patients (eg, patients 6, 7, 11, 13, 14, 17, 19, and 24) the results obtained with individual samples concurred markedly. Also the range of receptor levels varied considerably between these patients. For the eight patients for whom three or more samples were available, a coefficient of variation of 57 ± 28% (mean ± SD), ranging from 30% to 109% was found.

The nuclear androgen receptor content of biopsies showing hyperplasia was independent on the protein concentration of the nuclear extract (Fig. 4, upper panel). For biopsies from prostatic carcinoma, however, the receptor content of nuclear extracts containing more than 0.75 mg protein/ml was higher than that of extracts containing up to 0.25 mg/ml or 0.26 to 0.50 mg/ml, respectively (Fig. 4, lower panel).

No relation was observed between the nuclear androgen receptor level expressed per mg DNA and the DNA content of the nuclear pellets (Fig. 5). By contrast, for prostatic carcinoma biopsies a statistically highly significant correlation was observed between the nuclear androgen receptor content expressed per mg of DNA in the nuclear pellet and the nuclear androgen receptor content expressed per mg of protein in the nuclear extract (Fig. 6). This indicates the equivalence of the two methods for the expression of the results.

DISCUSSION

The results of the present investigation concerning the level of nuclear androgen receptors in normal prostatic tissue, BPH, and PC are in agreement with the findings of Bruchovsky et al [19] and Barrack et al [20]. The results are in contrast, however, with those of Trachtenberg et al [11] who found no difference between the nuclear androgen receptor content of hyperplastic and normal human prostatic tissue.

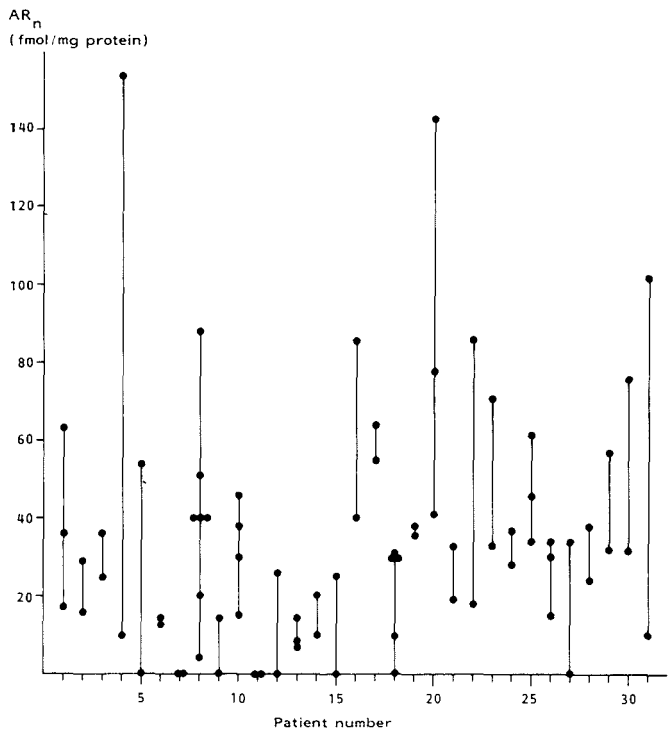

Fig. 3. Heterogeneity of nuclear androgen receptor content (ARn) of human prostatic carcinoma. Each point represents the assay result obtained from an individual sample composed of one or two biopsies. Assays done on different biopsies of the same patient are connected by vertical lines.

Barrack et al [20] attributed the possible reason for this contrast to methodological factors. Moreover, despite the observed lack of correlation between the androgen receptor content and the age of the patients (Fig. 2), a possible explanation for this contrast is that the age of the two groups studied by these authors differed considerably. The normal tissue in the study of Trachtenberg et al was obtained from patients aged 26 $\pm$ 3 yr, whereas BPH was obtained from patients aged 62 $\pm$ 2 yr. Fichmann et al [21] showed for the human foreskin, another androgen target organ, a relationship between the nuclear androgen receptor content and age-dependent physiological changes. If such an age-dependency also exists for prostatic tissue, it is imperative to use age-matched tissue donors, as in the present study. This is also illustrated by an experimental study of Trachtenberg et al [22] in which they observed a difference in nuclear androgen receptor content in age-matched young dogs with and without hyperplastic prostates.

In the BPH group a correlation was found between the percentage of epithelium and the nuclear androgen receptor content (Fig. 1). This could be explained, in theory at least, by a lower protein yield in nuclear extracts from BPH tissue containing a relatively small percentage of epithelium. The results in Figure 4 (upper panel) show, however, that for nuclear extracts form BPH tissue the ARn concentration does not depend on the concentration of protein in the nuclear extract. We have therefore

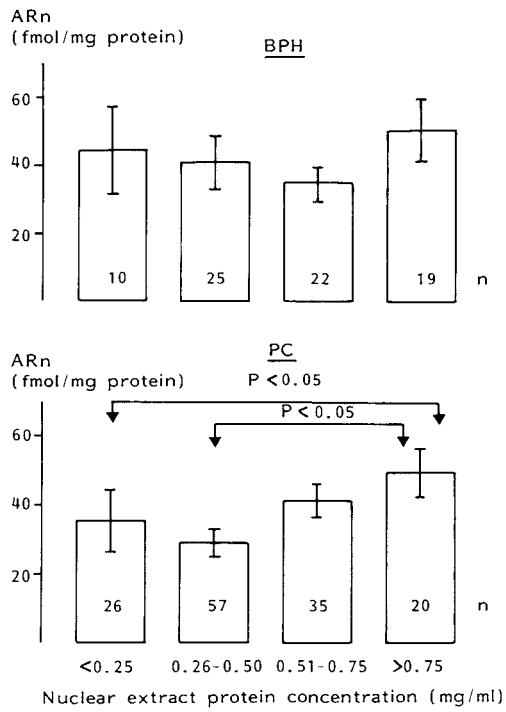

Fig. 4. Nuclear androgen receptor content (ARn) of prostatic biopsies showing hyperplasic (BPH) or carcicnoma (PC) as a function of the protein content of the nuclear extract. Results are given as means ± SEM; number within bar is number of samples.

concluded that differences in the percentage of epithelium cannot be used to explain the results in Figure 1. The results in Figure 1 suggest that the ARn level of epithelium is higher than that of the stromal compartment.

Patients with tumors of higher differentiation grades are known to have better prognoses than patients whose tumors are less well differentiated. Thus we expected to find a correlation between the histological grade of the tumor and its androgen receptor content. Surprisingly, however, such a relation was not found, which is in agreement with the results of Trachtenberg et al [14]. As reported earlier for large BPH specimens [18], in this investigation a considerble variation in nuclear androgen receptor content of multiple biopsies in the same prostatic carcinoma was also found (Fig. 3).

The extent of variation of ARn levels and the variable number of biopsies that can be obtained from the primary tumor raises the question whether the mean of these values reflects the true receptor content of the whole tumor load of a patient and whether the hormone dependence of the tumor is determined by the cells with the highest or lowest receptor content, respectively. Our results are in agreement with the finding of Isaacs et al [24] that even the Dunning R 3327-H rat prostatic carcinoma is composed of a mixture of preexisting clones of both androgen-dependent and -independent tumor cells, and this heterogeneity probably causes the variation found in receptor content when multiple biopsies of the same tumors are assayed. The

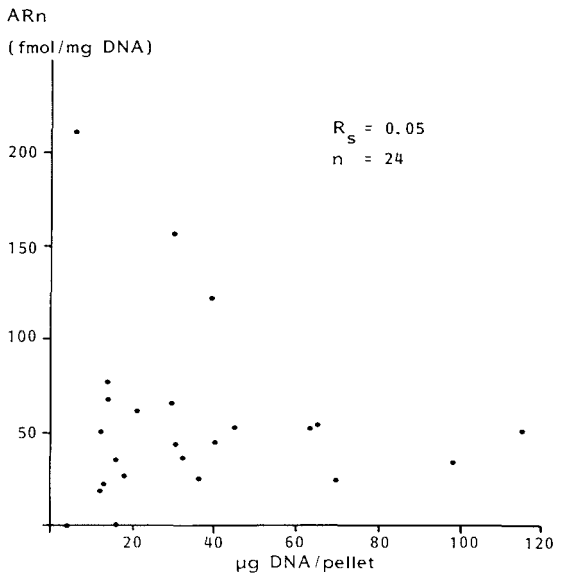

Fig. 5. Nuclear androgen receptor content (ARn) of prostatic carcinoma biopsies as a function of the DNA content of the nuclear pellet. No significant correlation was observed (R_s = 0.05; n = 24).

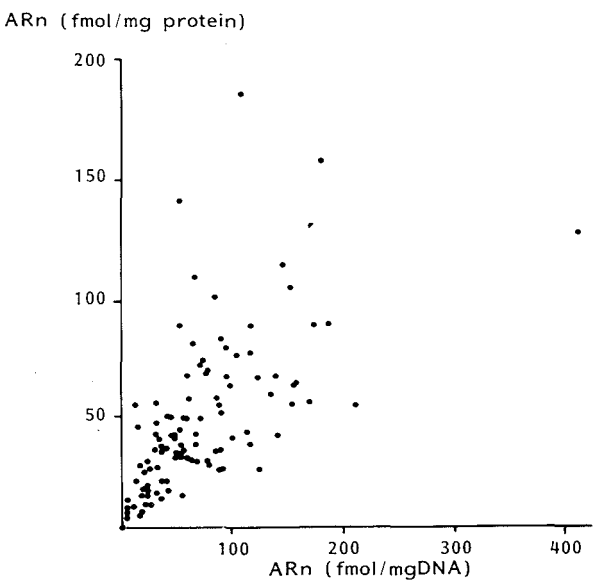

Fig. 6. Relationship between the nuclear androgen receptor content (ARn) of prostatic carcinoma biopsies expressed per mg of protein in the nuclear extract and expressed per mg of DNA in the nuclear pellet (R_s = 0.70; n = 140; $p < 0.001$).

results of Sluyser et al [25] with mammary tumors are noteworthy. They found that the growth behavior of heterogeneous mammary tumor grafts that contained more than 10% autonomous (hormone-independent) cells was essentially determined by these cells. It is questionable whether differences (eg, between 10 and 20% hormone-independent cells) can be detected on the basis of a receptor assay. The results of Ghanadian et al [13] and Trachtenberg et al [14] suggest that ARn can be used as a prognostic indicator.

We have observed that the performance of the protamine sulphate assay [18] and the nuclear androgen receptor content of BPH (Fig. 4, upper panel) are independent on the protein content of the sample. The finding that the nuclear androgen receptor content in prostatic cancer biopsies is dependent on the protent content of the nuclear extract (Fig. 4, lower panel) should, therefore, not be attributed to methodological factors but may be an intrinsic property of prostatic carcinoma. The finding that the nuclear androgen receptor content is independent of the DNA content of the nuclear pellet indicates that it may be advantageous to use this parameter for the expression of the results.

The receptors, however, are extracted from the nuclei. In our opinion, the parameter on which the result is expressed should bear a relationship to the extraction, and therefore we prefer to use the protein content of the nuclear extract for routine purposes. The validity of this approach is exemplified by the results shown in Figure 6. Regardless of how the results are expressed, a proper cut-off point between receptor-rich and receptor-poor tumors remains to be defined, especially when considering the discrepancy between Ghanadian et al [13] who use 500 fmol/mg DNA and Trachtenberg et al [14] who use 110 fmol/mg DNA as the limit for predicting progression. A prospective study to confirm the findings of Ghanadian et al [13] and Trachtenberg et al [14] has been started. At present, 82 patients are being followed.

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CHAPTER 5

PREDICTION OF TIME TO PROGRESSION AFTER ORCHIECTOMY BY THE NUCLEAR ANDROGEN RECEPTOR CONTENT FROM MULTIPLE BIOPSY SPECIMENS IN PATIENTS WITH ADVANCED PROSTATE CANCER.

OGJM van Aubel, J Bolt-de Vries, MA Blankenstein, and FH Schröder.

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The Prostate 12:191–198 (1988)

Prediction of Time to Progression After Orchiectomy by the Nuclear Androgen Receptor Content from Multiple Biopsy Specimens in Patients With Advanced Prostate Cancer

O. van Aubel, J. Bolt-de Vries, M.A. Blankenstein, and F.H. Schröder

Departments of Urology (O.v.A., F.H.S.) and Biochemistry (J.B.d.V.), Medical Faculty, Erasmus University, Rotterdam, The Netherlands; Department of Endocrinology, Medical Faculty, Utrecht, The Netherlands (M.A.B.)

The nuclear androgen receptor (ARn) content of cancerous prostatic tissue has been investigated as a prognosticator for time to progression under endocrine therapy. In 1981 a prospective study was started to investigate whether the ARn content in biopsy specimens of patients with prostatic carcinoma predicts the duration of response following hormonal treatment. ARn was estimated by a microassay which involves extraction of nuclear pellets with a heparin-containing buffer, exchange labeling of the nuclear extract with ^{3}H-R1881, and quantitation of the receptor with protamine sulphate precipitation. One hundred and fifteen patients with prostatic cancer entered this study; 47 patients had evidence of metastatic disease as proven by bone scan. Forty-two patients were treated by orchiectomy; 37 of these patients are evaluable with a minimal follow-up of 30 months. A relationship between the nuclear androgen receptor content and the time to progression following orchiectomy in these patients with metastatic disease of the prostate was not found. This could possibly be attributed to the heterogeneous nature of the prostatic tumor tissue with respect to the distribution of the ARn. We concluded that androgen receptor assay in needle biopsies, at least in this study, had no value for the prediction of the time to progression after orchiectomy.

Key words: exchange assay, prognosis, disease-free interval

INTRODUCTION

Although hormonal therapy has been the standard treatment for prostatic cancer for over 40 years, we are not able today to predict which patient will have a durable response to this treatment. Nearly all patients with advanced prostatic cancer treated by androgen ablation therapy do initially respond to some degree [1]. The duration of response to this therapy, however, varies among individual patients. It has been postulated that the reason for this variable response is due to clonal selection and or

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Address reprint requests to O. van Aubel, M.D., Department of Urology, St. Franciscus Hospital, Roosendaal, Boerhaavelaan 25, 4708 AE Roosendaal, The Netherlands.

selective overgrowth of hormonally independent populations of cells in prostatic carcinoma [2]. If the patient who will respond to androgen deprivation therapy for only a limited time could be identified before commencement of this therapy, chemotherapy alone or combined with hormone therapy could be started immediately, when the tumor burden is small and the patient may better tolerate this therapy. Steroid hormones exert their effect on target cells through steroid receptors. We have previously shown [3] that the nuclear androgen receptor (ARn) content of biopsy specimens was not different for BPH and prostatic carcinoma. Biopsies showing essentially normal prostatic tissue, however, had a lower ARn content. A considerable variation in ARn within tumors of individual patients was found; the content of ARn was independent of the age of the patients and of the histological grade of the carcinomas. For breast cancer the probability of response to endocrine therapy is related to the presence and concentration of estrogen and progestin receptors. The purpose of the present investigation was to evaluate whether the nuclear androgen receptor content of needle biopsies can be used to identify prostatic cancer patients who will respond to endocrine therapy for only a limited time.

MATERIALS AND METHODS

Between January 1981 and July 1984 a prospective study was done on the clinical value of nuclear androgen receptor estimation in patients with prostatic cancer. One hundred fifteen patients with prostatic cancer entered this study. Forty-seven had advanced disease, stage D_2 (TN_xM_1), proven by bone scan. None of these 47 patient had received any form of hormonal treatment before therapy. Patients ranged in age from 45 to 87 years. Before therapy all patients were carefully staged, by history, physical examination, and hematological and biochemical evaluation, including determination of serum testosterone and prostatic acid phosphatase and a bone scan. With informed consent, multiple perineal biopsies were taken under local anesthesia, one for histological diagnosis and two to four for receptor estimation. After staging, 42 of these patients were treated by bilateral subcapsular orchiectomy. Patients were evaluated at follow-up every 3 months by history, physical examination, and routine and specific laboratory examination; and every 6 months by x-ray of the chest and bone scan. Progression was defined as the appearance of new distant metastases seen on bone scan or x-ray or proven by biopsy, or increase in volume of the primary tumor proven by ultrasound or digital examination. Time to progression was defined as time between initation of hormonal therapy and relapse. The biopsies for receptor estimation were placed in liquid nitrogen immediately and stored at $-80°C$. Prior to the receptor assay a frozen section was made of each biopsy. Tissue showing severe signs of infection was excluded from this study, as was tissue showing less than 50% cancer cells.

Assay of Androgen Receptors

Androgen receptor assay was done essentially as described previously [3–5]. After taking a frozen section for histological diagnosis, the remainder of the tissue (25–50 mg) was placed in a miniaturized steel grid device consisting of a 10-cm-long stainless steel tube (8 mm in diameter) with a bottom of stainless steel gauze (80 mesh) and a stainless steel pestle covered with the same gauze. The tissue was

squeezed between the two layers of gauze while the entire device was immersed in 2 ml ice-cold 0.50 mmol/liter Tris HCl buffer, pH 7.5, containing 2.5 mmol/liter KCl, 5 mmol/liter $MgCl_2$ TKM buffer, and 0.55 mol/liter sucrose. The nuclei were concentrated and partially purified by layering the suspension over TKM buffer containing 0.88 mol/liter sucrose and centrifugation for 10 min at 1,000g. The nuclear pellet was resuspended in 10 mmol/liter Tris HCl buffer, pH 7.5, containing 1.5 mmol/liter EDTA, 1.5 mmol/liter dithiothreitol (TED-buffer), and 50 mmol/liter NaCl. For extraction of androgen receptor complexes, the nuclear pellet was suspended in 0.1 ml 2 mmol/liter phosphate buffer, pH 8.5, containing 1 g/liter heparin.

After 1 h, the suspension was centrifuged for 30 min at 100,000g, and the supernatant was termed the nuclear extract. The nuclear extracts were incubated in siliconized glass tubes at 10°C in the dark, in the presence of 10^{-8} mol/liter ^{3}H-R1881 (methyltrienolone, specific activity 87 Ci/mmol; New England Nuclear. Dreieich, FRG). Triamcinolone acetonide (5×10^{-6} mol/liter) was added to block the binding of ^{3}H-1881 to progestin receptors possibly present in the nuclear extract. Correction for aspecific binding was made by a parallel incubation in the presence of a 200-fold excess unlabeled R-1881. The incubation volume was 40 µl. The protamine sulfate precipitation assay was performed on 25 µl of the labeled extracts, and the precipitates were counted for 20 min in a Searle-type Isopac-300 liquid scintillation counter.

Other Procedures

The protein concentration of nuclear extracts were determined according to Peterson [6]. DNA was estimated in nuclear pellets with the method of Hinegardner [7].

RESULTS

Forty-seven of these 115 patients with prostatic carcinoma had bone metastases and elevated prostatic acid phosphatases; 42 patients were treated by bilateral orchiectomy, the other 5 patients were excluded from this study because 2 received no therapy and 3 were treated by other forms of hormonal therapy. At present 37 patients are evaluable with minimal follow-up of 30 months: 25 had progression and all 25 are now deceased. The end point of the study was time to progression; a comparison was made between time to progression and the nuclear androgen receptor content (ARn) of individual biopsies. The result is shown in Figure 1. The time to progression of the disease appears not to be related to the ARn level of individual biopsies. When the amount of tissue exceeded 50 mg, more than one receptor estimation was done; a considerable variation of receptor content was found. It can be envisaged that the ultimate behavior of a tumor is determined by that part which is differentiated most, or, alternatively, that response to endocrine therapy is determined by the presence of a relatively receptor-poor area. We looked for a correlation between time to progression and highest or lowest receptor content. The data in Figure 2 show that such relationships do not occur. In published reports [8–12] different cutoff values have been used to divide patients with prostatic carcinoma into two groups, one with high receptor levels and a good prognosis and one with lower

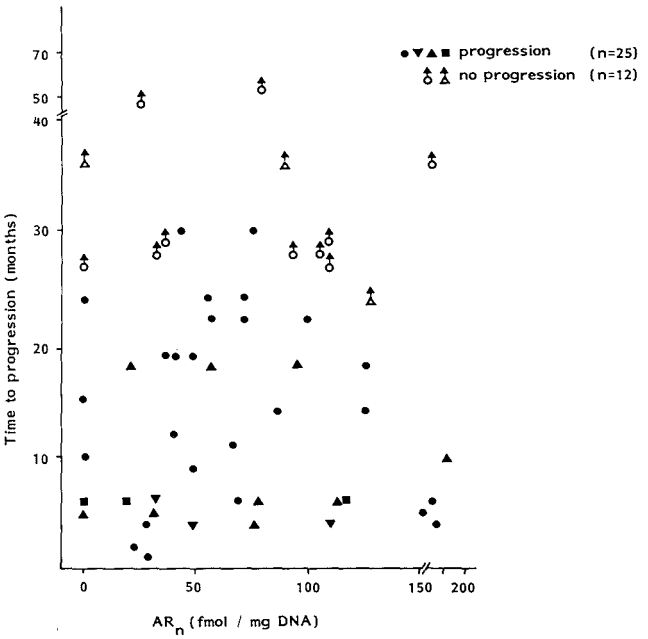

Fig. 1. Time to progression as a function of the nuclear androgen receptor (ARn) content of individual needle biopsies. When several patients had the same time to progression, receptor values of each patient are given by individual symbols.

receptor values and a relatively poor prognosis. The data of this study have also been analyzed in such a way, using different cutoff levels. It was not possible to identify two groups of patients with a significantly different time to progression (Fig. 3). In four patients with clinically localized prostatic carcinoma who underwent a staging lymphadenectomy and proved to have nodal disease, the ARn content of the positive nodes was measured, and different values for the primary and its associated metastatic tissue were found (see Fig. 4). Also in the nodal tissue, a high variation in ARn was observed.

DISCUSSION

In the present study it was shown that the ARn content in needle biopsies of cancerous prostatic tissue was of no value for the prediction of time to progression after androgen withdrawal. In contrast to other studies [8–12] with relatively small numbers of patients, in which patients were not uniformly treated and in which follow-up periods were much smaller, all 42 patients in this study were uniformly treated by orchiectomy and all had a minimal follow-up of 30 months.

Once progression occurs on hormonal therapy no common opinion exists on further treatment, second-line hormonal therapy or chemotherapy, or no treatment. For this reason only time to progression and not survival was used as an end point. We could not confirm the putative correlation between ARn content and duration of

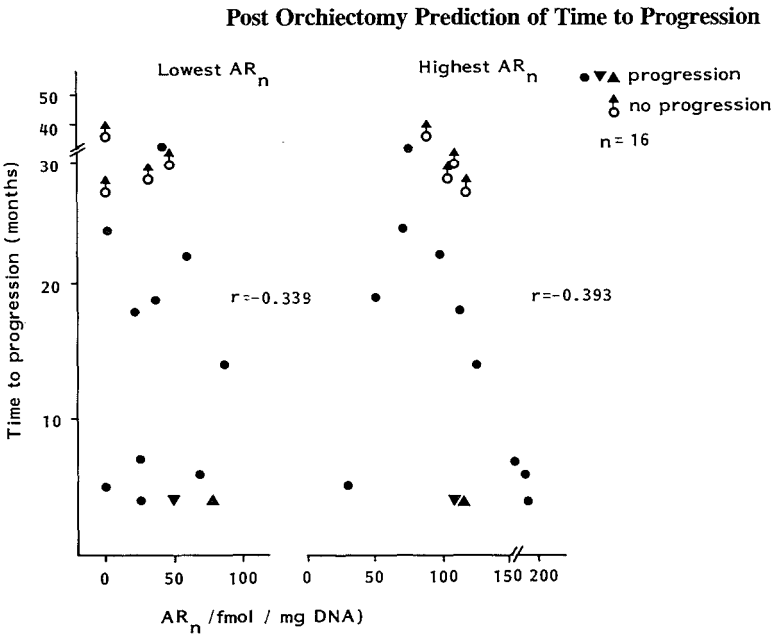

Fig. 2. Time to progression according to lowest or highest ARn found in multiple needle biopsies.

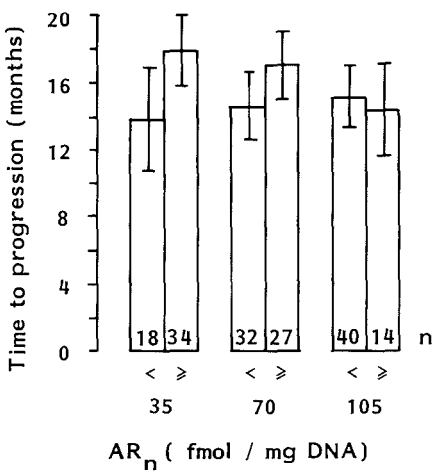

Fig. 3. Time to progression of advanced prostate cancer as a function of the nuclear androgen receptor level prior to orchiectomy. Results are given as means ± SEM; n = number of observations.

response found by others [8–12]. Although methodological differences in the estimation of the ARn could be an explination for these differences, approximately the same ARn levels as those found by other authors using similar techniques [8–12] were found. Since patients with advanced disease are not subjected to open surgery, material has to be obtained by needle biopsy. However, prostatic carcinoma is

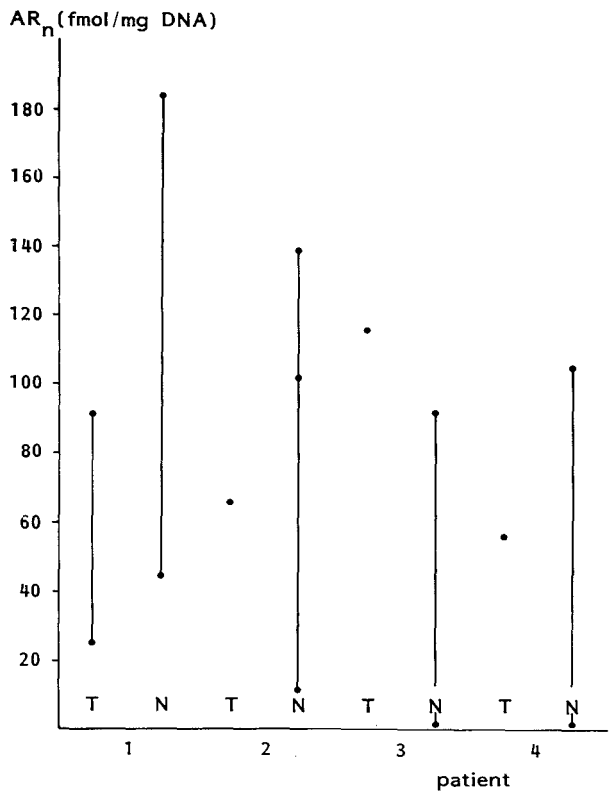

Fig. 4. Heterogeneity of ARn of human prostatic carcinoma in the primary tumor (T) and positive lymph nodes (N) of the same patient. Each point represents the assay result obtained from an individual sample.

histologically heterogeneous; varying degrees of differentiation can often be seen in one and the same tumor. Müller et al. [13] demonstrated that needle biopsies are not always representative for the degree of differentiation of a tumor. In a previous study [3], a considerable variation of receptor content within tumors of individual patients was demonstrated when more than one receptor estimation was done. The extent of variation in ARn levels and the variable numbers of biopsies that can be obtained from the primary tumor raises the question of whether the means of these values reflect the true receptor content of the whole tumor load of a patient (i.e., primary lesion and also secondary lesions). Benson et al. [14] found different values of androgen binding for the primary and its associated metastatic tissue. In four patients who underwent a pelvic lymphadenectomy for staging of their prostatie cancer in the present study, the receptor level of the primary and positive nodes were measured. Differences between the primary tumor and the metastases comparable in magnitude to those reported by Benson et al. [14] were observed. Benson et al. [14] found that androgen binding results obtained from a single-point analysis performed on needle-biopsy specimens (about 50 mg) of the prostate correlated poorly with those

derived from a full six-point Scatchard analysis performed on large samples (500–1,000 mg) removed from the center of the malignancy. For the receptor assay used in the present study it has been shown that the mean ARn content was not dependent on the amount of tissue used (25 mg, 50 mg, 100 mg, 500 mg). We have previously reported on the validity of this single-point assay for homogenized tissue pools [5]. For actual needle biopsies a large variation in receptor values in the same tumor was found. In contrast to Benson et al. [14], we attribute these findings to the extensive heterogeneity of the tissue. Moreover, when this method was used for the transplantable prostatic tumor model PC 82 the intratissue coefficient of variation for the ARn assay was found to be approximately 20% [15]. The finding of Isaacs et al. [16] that prostatic carcinoma is composed of a mixture of preexisting clones of both androgen-dependent and -independent tumor cells is in line with the conclusion that the extent of variation of ARn levels is based on the heterogeneity of tissue and not on this assay method nor the amount of tissue used. Assuming the concept of clonal selection and selective overgrowth of hormonally independent cells as the only mechanism of hormone-independent growth of prostatic carcinoma, we looked for a correlation between the lowest receptor content that may reflect the hormone-independent cells and time to progression. Such a correlation was not found (see Fig. 3). Trachtenberg and Walsh [8] were the first who reported a relationship between the ARn and the duration of response and survival following hormonal treatment in patients with advanced prostate cancer. Empirically they segregated their patients in those with low and high receptor levels and found a cutoff point of 110 fmol/mg DNA. To allow a proper comparison of these results with published data, three cutoff points—35, 70, and 105 fmol/mg DNA (Fig. 3)—were studied. A cutoff point which enabled us to separate our patient group into those with a shorter or longer time to progression was not found.

Later Brendler et al. [17] found that an index based on multiple biochemical variables (including six enzymes, androgen receptor, and tissue steroid measurements) separated the two response groups better than a single variable alone. Sluyser et al. [18] found in mammary tumor grown in vivo that the growth behavior of heterogeneous tumor grafts that obtained more than 10% autonomous (hormone-independent) cells was essentially determined by these cells. So it is questionable whether differences (e.g., between 10% and 20% hormone-independent cells) in an already heterogeneous tumor can be detected on the basis of a receptor assay. The main conclusion of the present study is that the heterogeneity of prostatic tissue with respect to the distribution of ARn and the possible presence of nonmalignant prostate tissue precludes the use of biochemical androgen receptor assays on perineal biopsies for evaluation of the putative value of ARn. This does not eliminate the possibility that androgen receptors might be of value in predicting duration of response if assayed differently. More refined techniques are required to attack this problem. Such techniques should be able to demonstrate the heterogeneity of the sample and to assess the presence of receptors in single malignant cells. Autoradiographic or immunocytochemical localization of the androgen receptor may prove of great value in this respect. Recent results on the use of autoradiography [19] seem promising. For immunocytochemical assays monoclonal antibodies against the human androgen receptor have to be developed. As long as these do not become available, circulating antibodies against the human androgen receptor [20] may be of value.

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CHAPTER 6

CIRCULATING TESTOSTERONE, PROSTATIC NUCLEAR ANDROGEN RECEPTOR AND TIME TO PROGRESSION IN PATIENTS WITH METASTATIC DISEASE OF THE PROSTATE TREATED BY ORCHIECTOMY.

OGJM van Aubel, J Bolt-de Vries, MA Blankenstein, FH de Jong and FH Schröder.

This chapter will be published: Urol. Research, 17 (1989)

SUMMARY

The content of nuclear androgen receptors (ARn) in prostatic carcinoma biopsies is not predictive for the duration of response of the tumor to endocrine therapy (Prostate 1988, 12: 191-198). Recently pre-treatment plasma testosterone has been suggested to be predictive in this respect (J Urol 1986, 136: 1038-1040). Therefore, pre-treatment plasma testosterone (T) and sex hormone binding globulin (SHBG) levels were studied in 31 patients aged 72 $\pm$ 10 years (range: 45-87) with stage D2 carcinoma of the prostate treated by orchiectomy. In 26 of these patients, the ARn level of the carcinoma was also known (61 $\pm$ 41 fmol/mg protein; range 0-169). Plasma T levels (mean: 13.7 $\pm$ 6.1 nmol/l) varied widely (range: 2.4-25.4), as did plasma SHBG (32.5 $\pm$ 19.3 nmol/l; range 4.4-78.8), and time to progression (TTP; 14.6 $\pm$ 11.2 months; range 1 - 48). Plasma T was found to be correlated to age (R_s = 0.537; $P<0.01$) and TTP (R_s = 0.4495; $P<0.02$). Tissue ARn and plasma SHBG did not correlate to any of the parameters studied.

INTRODUCTION

Huggins and Hodges [1,2] in 1941 established the scientific basis for the androgen dependence of most prostatic cancers and demonstrated the benificial effects of orchiectomy or oral estrogens in most patients with metastatic disease. The degree and the duration of response to androgen deprivation,

however, are variable: 10 percent of patients die within six months, 50 percent of patients have a survival of less than 3 years and only 10 percent are still alive after 10 years [3]. Identification of patients, who will benefit only for a limited time, before initiation of androgen deprivation therapy might allow earlier institution of alternative treatment forms.

About 97 percent of testosterone in plasma is bound to proteins, sex steroid binding globulin (SHBG) and albumin; less than three percent is unbound. Generally, only the free testosterone is considered to be functionally active. Androgens exert their effects on target tissues through androgen receptors. We have shown that the content of nuclear androgen receptors (ARn) in biopsy specimens of prostatic carcinoma is not predictive for the duration of response of the tumor to endocrine therapy [4]. Recently the pre-treatment plasma testosterone concentration has been suggested to be predictive in this respect [5]. Therefore, the relationships between prostatic nuclear androgen receptor levels and pre-treatment serum testosterone, calculated free testosterone and SHBG levels and the time to progression after orchiectomy in patients with stage D2 carcinoma of the prostate were evaluated in an attempt to identify those patients whose carcinoma would soon escape androgen suppression.

MATERIAL AND METHODS

Patients and Tissue

Between January 1981 and July 1984 a prospective study was done on the clinical value of the nuclear androgen receptor estimation in patients with prostatic cancer [4]. One hundred and fifteen patients with prostatic cancer of all stages entered this study. Fourty-seven had advanced disease stage D2 (TNxM1) proven by bone scan. None of these patients had received any form of hormonal treatment before therapy. The age of the patients ranged from 45 to 87 years. Before therapy all patients were carefully staged, by history, physical examination, hematological and biochemical evaluation, and a bone scan. Patients gave informed consent and multiple perineal biopsies were taken under local anesthesia. Before taking the biopsies a 10 cc blood sample was drawn for steroid measurements. Fourty-two of these 47 patients with stage D2 carcinoma of the prostate were treated by bilateral subcapsular orchiectomy. Thirty-one patients are evaluable with a minimal follow up of 30 months. Of 26 of these patients, the ARn level of the carcinoma tissue are known. Patients were evaluated at follow-up three-monthly by history, physical examination, routine and specific laboratory examination and six-monthly by X-ray of the chest and bone scan. Progression was defined as appearence of new distant metastases on bone scans, or X-rays preferably proven by biopsy, or increase in volume of the primary tumor proven by ultrasound or digital examination. Time to

progression was defined as time between initiation of hormonal therapy and relapse. The biopsies for the receptor estimation were placed in liquid nitrogen immediately and stored at -80 C. Prior to the receptor assay a frozen section was made of each single biopsy. Tissue showing severe signs of infection was excluded from this study, as was tissue showing less than 50% cancer cells.

Assay of androgen receptors

The method used has been described in detail elsewhere [6,7] and involves extraction of nuclear pellets with a heparin containing buffer, exchange labelling of the receptors with 10-8 mol/liter 3H-R1881 at 10¦C in the presence of a 500-fold excess of triamcinolone acetonide, and quantification of the receptors following protamine sulphate precipitation. Correction for aspecific binding was made by a parallel incubation in the presence of a 200-fold excess unlabelled R-1881. The protein concentration of the nuclear extracts was determined according to Peterson [8]. DNA was estimated in nuclear pellets with the method of Hinegardner [9].

Steroid Measurements

Plasma testosterone was measured by radioimmunoassay as described before [10]. Sex hormone binding globulin (SHBG) in plasma was determined using the method described by Hammond et al [11]. Free testosterone was calculated from total testosterone and SHBG according to the method of

Vermeulen [12]. In this calculation plasma albumin was taken to be 50 g/l. This was done to avoid errors inherent to retrospective measurement of albumin. The existence of correlations between the parameters was tested by Spearman's rank correlation test.

RESULTS

The data obtained are summarized in Table 1. In the entire group of patients studied, a correlation between time to progression and ARn was not observed [4]. In the present subgroup of 26 of these patients the average ARn level of the carcinoma tissue was 61 $\pm$ 41 fmol/mg protein: range (0 - 169). Plasma T levels (mean: 13.7 $\pm$ 6.1 nmol/l) varied widely (range: 2.4-25.4), as did plasma SHBG (33.5 $\pm$ 19 nmol/l; range 4-79), and time to progression (TTP; 14.6 $\pm$ 11.2 months; range 1 -48).

Statistically significant correlations were found between plasma T and age (Rs = 0.537; $P<0.01$), figure 1, and between plasma T and TTP (Rs = 0.4495; $P<0.02$) figure 2. Tissue ARn and plasma SHBG did not correlate to any of the parameters studied (Table 2). No advantage was noted in calculation of the apparent free testosterone concentration.

DISCUSSION

The role of testosterone in the maintainance and growth of the prostate is well established [13,14]. Cancerous prostatic tissue appears to be androgen dependent to a certain

Table 1. Overall results of measurements in patients with advanced prostatic carcinoma.

parameter*	n	Mean	SD	Range
Age (y)	31	72	10	45- 87
TTP (months)	31	19.6	11.2	1- 48
ARn (fmol/mg p)	26	61	41	0-169
Plasma T (nM)	31	13.7	6.1	2.4-25.4
Plasma SHBG (nM)	31	32.5	19.3	4- 79
"Free T" (nM)	31	0.29	0.13	0.05-0.56

*Abbreviations used: TTP: time to progression; ARn nuclear androgen receptor; T: testosterone; SHBG: sex hormone binding globulin; "Free T": calclulated free testosterone concentration.

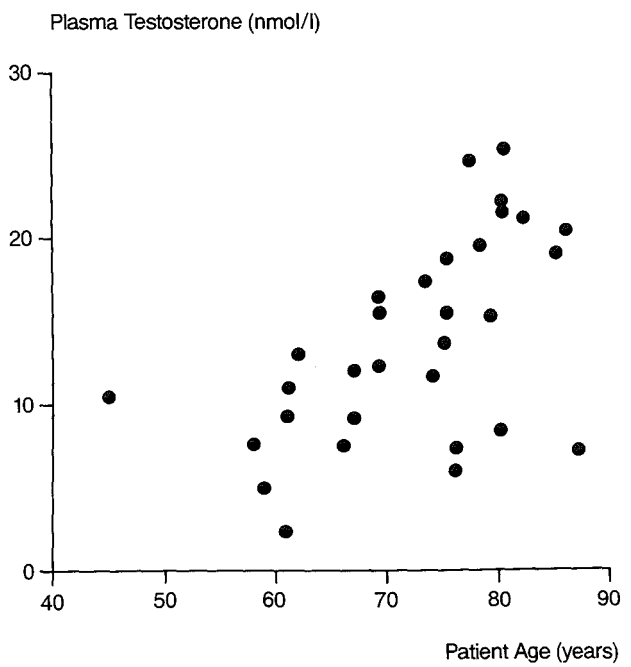

Figure 1. Pretreatment plasma testosterone versus age in men with advanced prostatic carcinoma. (Rs = 0.5371; P<0.01; n=31).

Table 2. Spearman Correlation Coefficients (Rs) between tissue Androgen Receptors (AR), plasma testosterone (TESTO), SHBG, calculated free testosterone ("FREE T") and time to progression (TTP) following endocrine therapy.

	AR	TESTO	TTP	SHBG	"FREE T"
AGE	0.0551	0.5371***	0.2176	0.2966	0.4050*
AR		0.1414	0.1755	0.0616	0.2445
TESTO			0.4495**	0.3302	0.8556***
TTP				0.0998	0.3577
SHBG					-0.0827

* P<0.05; **P<0.02; *** P<0.01

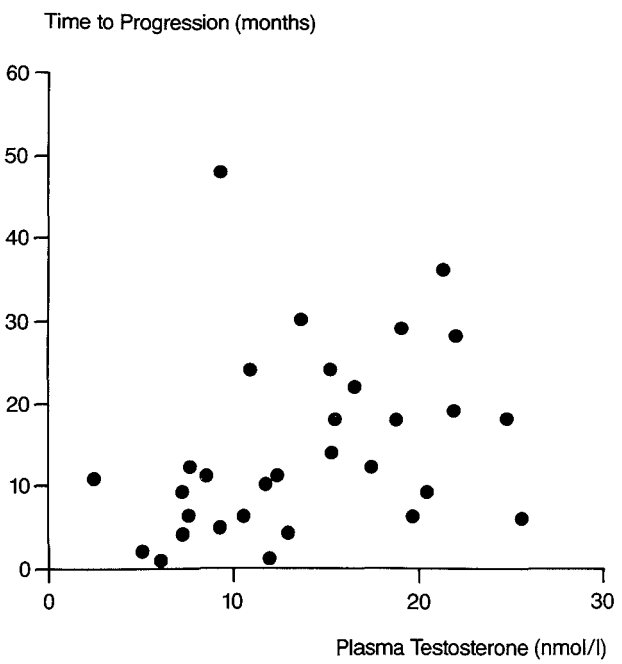

Figure 2. Time to progression after endocrine treatment versus plasma testosterone. (Rs = 0.4495; P<0.02; n=31).

extent, since many prostatic cancer patients initially respond to endocrine therapy. The duration of this response is variable [3].

Most studies on plasma testosterone levels in patients with prostatic desease are aimed at finding a difference in testosterone levels between patients with prostatic cancer and those with benign hyperplasia or a normal prostate [15-17]. Other studies are aimed at finding a relationship between plasma testosterone and stage [18,19] and grade [18]. In this study we evaluated the relationship between pre-treatment plasma testosterone, SHBG, and calculated free testosterone, to needle biopsy ARn and the time to progression in patients with stage D2 prostatic cancer after orchiectomy. Only pre-treatment plasma testosterone was found to be correlated to time to progression.

Other studies (5,19-23) have used less well-defined end points such as response or survival. In spite of this difference, our results agree with these findings.

In addition, we studied the relationship between plasma SHBG and testosterone and needle biopsy androgen receptor levels. No such relationships were found. The existence of a relationship between pretreatment plasma testosterone and a well-defined criterium, time to progression, as well as less well defined parameters, response and/or survival, adstructs the value of plasma testosterone as a prognostic indicator.

Normally, plasma testosterone levels cover a wide range. Serum testosterone levels in large populations of healthy men decline with age in some studies [24] but not in others

[25]. Following castration there is a marked depression of serum testosterone [26] resulting in testosterone levels in the female range (0.5 - 3 nmol/l). In this study 7 patients had serum testosterone levels already below 8 nmol/l before androgen deprivation. In all these 7 patients the tumor progressed within 12 months after castration. These patients had a low serum testosterone already at the beginning of their malignant disease, or the serum testosterone was lowered as a result of deterioration of the patient. In either case patients presenting with low serum testosterone at the time they are diagnosed with stage D2 carcinoma of the prostate might be considered to have experienced already a period of androgen withdrawal before diagnosis. These patients are already relapsing, when they are diagnosed as a result of outgrowth of their androgen independent tumor cell population.

It is tempting to speculate that the success of endocrine therapy of prostate cancer depends on the degree of suppressibility of plasma testosterone that can be obtained. We suggest therefore to measure plasma testosterone prior to the start of androgen suppressive therapy, in order to obtain an indication of the probable effect of this treatment.

ACKNOWLEDGEMENT

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CHAPTER 7

GENERAL DISCUSSION

7.1 Introduction

Since 1941, when Huggins and Hodges (1) demonstrated that prostatic cancer is dependent on continuing androgenic stimulation, hormonal therapy aimed at androgen deprivation of the prostate became widely used. Initially hormonal therapy by estrogens was used in every stage of the disease. Later on, because of the side effects, estrogen administration was confined mainly to metastatic disease. Endocrine therapy does not cure a patient of his prostatic carcinoma but only inhibits tumor progression for various periods of time. Thus 10 percent of the patients die within 6 months, 50 percent survive more than 3 years and only 10 percent live more than 10 years after diagnosis. When these data became available (2,3) therapy was generally witheld until the patient became symptomatic. Today, we are unable to predict the duration of response to hormonal treatment of individual prostatic tumors. If we could identify patients who were unlikely to experience a prolonged response to hormonal therapy, these patients might be given chemotherapy at this early time rather than delayed until the patient is symptomatic from metastatic disease and therefore probably less able to tolerate cytotoxic drugs. Effective chemotherapeutic

agents, however, are not available yet (4,5).

The presence of estrogen and progestin receptors in tumor tissue has been shown to be related to the prognosis of patients with breast cancer. By analogy, studies on a possible relationship between the androgen receptor content in prostate tumors and the response to endocrine treatment were started.

The aim of the study reported in this thesis was to investigate the occurrence of nuclear androgen receptors in biopsies from human prostatic tissue and a possible relationship between the presence or concentration of the ARn and the duration of response to endocrine treatment in patients with stage D2 carcinoma of the prostate. It was concluded, that the measurement of ARn content in multiple biopsies has no value in predicting time to progression in stage D2 patients after orchiectomy. Tumor cell heterogeneity and contamination by nonmalignant prostatic tissue may account for the difficulties in using receptor assays on homogenates. For this reason progress might only be expected from methods who take into account the tissue heterogeneity.

7.2 Steroid receptor assays as predictive test for endocrine therapy

Based on the studies of Jensen (6) the concept of specific hormone receptors in endocrine target tissues, especially the breast tumor estrogen receptor (7) was extensively explored. For breast cancer the probability of response to endocrine therapy was highly correlated to presence of these

receptor complexes (8). Prompted by these observations the prostate was investigated to determine whether or not androgen receptors were present in this target tissue and if so, whether their presence or content were related to the response of prostatic cancer to hormonal therapy. A variety of techniques to measure androgen receptors in prostatic tissue, has been developed. In a review (9) Menon concluded, that the sucrose density gradient centrifugation, ion exchange chromatography, and the protamine sulphate precipitation assays provide specific means for the identification of AR in human prostatic tissue. The androgen binding measured by these techniques is saturable and shows a high affinity, tissue specificity, and steroid specificity.

The first clinical data were presented by Wagner et al (10), who concluded that no correlation existed between cytosolic AR concentration and response to hormonal therapy. In the same year Mobbs et al (11) reported a positive correlation between receptor level and response to hormonal therapy. The major drawback of these two studies is that the measurements were made after the patients were subjected to therapy. De Voogt et al (12) reported that the DHT receptor value in 21 patients who had not been treated before was not related to response to endocrine therapy. By contrast Gustafsson et al (13) found a positive correlation in 25 prostatic cancer patients of all stages treated by orchiectomy or with estrogen. In these early reports the AR was measured in the cytosol from relatively large tissue samples. After Shain et al(14) and Walsh et al (15) demonstrated that 72% of the

total androgen receptor was localized in the nucleus, and because steroid hormones exert their major influence within the nucleus of target tissues, further studies were focused on individual cytosolic and nuclear compartments (16-19). In addition miniaturisation of the methods was persued. Reliable assays which were sufficiently miniaturized to enable the measurement of AR on tissue specimens obtained by needle biopsies were developed by Hicks et al (20) and Blankenstein et al (21).

The total AR content (cytosolic and nuclear) was reported to be correlated to response (17,19), Trachtenberg and Walsh (16) did not find this correlation, they found only a positive correlation between duration of response and the AR content of the nucleus. We did not find such a relationship in our material.

Barrack and Coffey (22,23) demonstrated that in the rat prostate nuclear matrix bound androgen receptors, which are resistant to high ionic strength buffers, may be the primary determinant of androgen action. Fentie et al (24) and later Gonor et al (25) studied the concentration of nuclear matrix-bound androgen receptor in human prostate. They both reported a correlation between extractable and non-extractable ARn and response to hormonal therapy.

Characteristics of the different studies are summarized in table 7.1. The objections to these studies are the small number of patients (8 - 24), the use of patients with different stages of disease the use of different treatment modalities (orchiectomy, orchiectomy plus estrogen or anti-

androgen or estracyt), but above all insufficient follow up time (4 - 38) months.

In cancer treatment it is extremely important that rigid criteria are applied in defining response to therapy. Responses must be assessed as objectively as possible. In studies on the validity of receptor assays survival, or response, should not be studied, but time to progression. Survival in prostate cancer patients is influenced by non cancer related death and second line hormonal or chemotherapy today. In many studies (17,24,25) response or survival were studied, but rigid criteria for evaluating a response were not given. Objective response is extremely difficult to assess and the criteria have undergone continuous evolution (26, 27) during the last 5 years. In the present prospective study time to progression was studied as endpoint, progression was defined as - appearence of new metastases on chest ray or bone scan, - elevation of previously normal prostatic acid phosphatase level on 2 consecutive follow up visits, - appearance of biopsy proven soft tissue metastases, or increase in volume of the primary tumor (measured by ultrasound, or evaluated by digital examination).

These were accepted criteria of response at the beginning of this study. In phase III trials in advanced disease the NPCP and EORTC GU group use revised criteria (26,27). The most important change is that the increase in acid or alkaline phosphatase alone is not to be considered as an indication of progression but should be used in conjunction with other criteria. Considering the differences between the definition

of progression used in our study and the NPCP criteria for progression, it is important to note that we found time from progression to death to be approximately the same (3 - 27 months) as reported Trachtenberg and Walsh (16) who used the NPCP criteria in a similar study.

An other important issue in prostatic cancer studies is the minimum time for which the patients have to be followed in order to have findings of value. From the study of Blackard et al (28) it is known that the degree and duration of response after orchiectomy in patients with advanced prostatic cancer are variable. Ten percent of patients live less than 6 months, 50 percent survive less than 3 years and only 10 per cent live more than 10 years. In the present study time from progression to death was 8.9 $\pm$ 6.3 months (mean $\pm$ SD, n = 20) and in the study of Trachtenberg (10.1 $\pm$ 4.9 months, n = 23). For a proper evaluation of time to progression completion of data for at least 50% of the patients seems desirable. Taking into account a general median survival time of 36 months and a time from progression to death of 7-10 months (present study and Trachtenberg) a minimal follow up time of at least 26 - 29 months for all patients appears to be required. Based on these criteria most of the studies mentioned in Table 1 are deficient.

In our study the 25 patients who had progression, progressed before 30 months, the 7 patients who are stable until now have all passed this period of time. Taking into account the calculated minimal follow-up time of 26 months as require-

Table 7.1. Summary of published studies on the prognostic significance of nuclear androgen receptors in carcinoma of the prostate

authors	n	stage	tissue collection	therapy	parameters studied	definition of criteria	follow up in months	conclusion
Ghanadian et al (81)	32	not mentioned	Tur	orchiectomy estrogens, estracyt cyproterone acetate	response	+	6 - 24	positive correlation
Trachtenberg et al (82)	23	D_2	needle biopsy	orchiectomy estrogens	time to progression survival	+	13 - 38	positive correlation
Brendler et al (84)	16	D_2	needle biopsy	orchiectomy estrogens	time to progression	+	13 - 29	positive correlation
Gonor et al (84)	13	D_1, D_2	Tur cold punch	orchiectomy estrogens	duration of response	NPCP	6	relationship
Larminat et al (86)	13	not mentioned	needle biopsy	orchiectomy estrogens	response	—	4 - 12	relationship
Fenti et al (86)	12	D_2	cold punch	orchiectomy estrogens	survival	NPCP	18 - 29	positive correlation
Benson et al (87)	9	D_2	cold punch	orchiectomy estrogens	time to progression	NPCP	14 - 31	relationship
Gorelic et al (87)	27	D_1, D_2	needle biopsy lymphadenectomy	orchiectomy estrogens	response survival	—	5 - 52	no relationship
van Aubel et al (88)	37	D_2	needle biopsy	orchiectomy	time to progression	+	30 - 52	no relationship

ment for receptor studies all data published so far are only preliminary and must be considered with great caution. Despite the limited follow-up period in the study, reported by Trachtenberg and Walsh (16) a significantly higher mean duration of response and mean survival time were observed for patients whose tumors contained more androgen receptors than 110 fmol/mg DNA than for those whose tumors had lower receptor concentrations. There was considerable overlap in response times in these two groups, suggesting that nuclear ARn may be a valuable factor for prognosis and indicator for treatment, but must be supplemented by other information. In a second report by Brendler et al (29) in which 6 enzymes were measured, androgen receptor content and tissue testosterone and dihydrotestosterone content, in tissue from 26 patients, it was concluded that an index based on multiple

enzyme activities, separated the responders and non-responders better than any single variable alone. Moreover when salt extractable nuclear androgen receptor was included in the numerator of this index the 2 groups were separated almost completely. This finding is potentially of interest, however the procedure is complex and therefore probably not suited for clinical routine.

7.3 Factors affecting the result of AR measurement in prostatic tissue

The result of ARn measurement in prostatic biopsies may be influenced by the presence of nonmalignant parts in the biopsies, the different percentage of cell types, and the collection of tissue.

Normal human prostate can probably only be found in males aged 15 - 35 years. The prostate of eldery men usually shows many alterations characteristic of senescence, such as partial atrophy sclerosis and cyst formations (30,31). In order to find differences in AR between normal and pathologic prostates it is therefore important to use age matched controls or better different parts of a single prostate with apparently "normal" or diseased parts. Early benign hyperplasia is histologically difficult to distinguish from normal prostate. Therefore comparisons of assay results from "normal" and pathological prostate tissue must be made with great caution. The results reported in this thesis for "normal prostate" are from radical prostatectomy specimens obtained from patients of the same age. We found (Chapter 4)

statistically significant differences between "normal" and pathologic prostatic tissue with respect to the androgen receptor content. The conclusion, that the ARn content of "normal" prostatic tissue is significantly lower than the Arn content of pathologic tissue and that there is no significant difference between ARn content of hyperplastic and carcinomatous prostatic tissue can compromise the results of receptor assays obtained when using tissue homogenates. In a morphometric analysis Bartsch et al (32) showed that biopsy specimens of 50 patients with prostatic carcinoma contained 23% cancer cells and 72% stromal tissue and normal or hyperplastic glandular cells in 5% of all biopsy specimens: this morphometric analysis shows the heterogeneity of prostatic biopsy specimens in respect to different percentage of cell types. Microscopic analysis of each biopsy is therefore mandatory to support the receptor assays. Therefore in this study a histopathologic diagnosis was done of each single biopsy, and only biopsies showing more than 50% cancer cells were used. Other investigators have not reported detailed information on the composition of the biopsy specimens used. The collection of tissue might also affect the apparent presence of AR in prostatic tissue. Most receptor studies used large amounts of tissue 500 - 1000 mg, obtained by TUR, cold punch biopsy or open procedures. The instability of the receptor at higher temperatures (33) make that electro-resected specimens are generally considered as unreliable for receptor quantitation. Some authors (34,35) however, claim that TUR-specimens can be used for assay of

androgen receptor. This issue, therefore, remains controversial.

Most patients with stage D2 disease do not require prostatic surgery. In this respect the only acceptable manner to collect tissue from the prostate is by needle biopsy. Hicks and Walsh (20) and later Blankenstein et al (21) established micro assays for ARn that may be carried out on 25 - 200 mg of tissue obtained by multiple needle biopsies. Six to eight biopsies are required to obtain approximately 200 mg of tissue. In view of the discomfort to the patient this number of biopsies should not be exceeded.

7.4 The relationship between nuclear androgen receptor content the grade of the tumor and the stage of the disease

The grade or differentiation of a tumor, which indicates the degree of retention of the normal prostatic morphology is related to the cancer death rate (36-38). The extent of the tumor or stage at the time of diagnosis has also proven to be a potential mean of predicting the natural history and the course under treatment of prostate cancer in individual patients (39,40).

The relationship between tumor grade (41-46) and stage (45) and the AR content of the tumor has been studied previously by several authors.

In this study the grade of the tumor was determined according to the Mostofi grading system (46) which takes into consideration nuclear pleomorphism as well as the changes of

the glandular pattern. Grade 1 indicates a well differentiated, grade 2 a moderately, and grade 3 a poorly differentiated tumor. Before therapy all patients were staged according to the tumor, node, metastases (TNM) system of 1978 - 1982 (47), by history, physical examination, haematological and biochemical evaluation and a bone scan.

In figure 2 of Appendix Paper II the content of ARn for prostatic carcinoma biopsies of different histological grade is given. The ARn content of G1 prostatic carcinomas appeared to be significantly lower than that of tumors of other histological grades.

Tumor grade in prostatic cancer patients correlates with cancer death rates (36-38). Patients with tumors of higher differentiation grades are known to have a better prognosis than patients whose tumor is less well differentiated. Some investigators (41,42,46) found a positive correlation between the grade and ARn, others (43,44) did not find such a correlation. We found G1 tumors to have a lower ARn content than the less well differentiated tumors. This finding is opposite to what might be expected since patients with higher differentiation grades are known to have a better prognosis and therefore might have a higher ARn content than the patients whose tumor is less well differentiated. The different number of observations between G1, G2, G3 and the difficulty in finding biopsies in which only one type of differentiation was present might have influenced the result. It seems not unrealistic to suppose that the other studies mentioned may also have suffered from this drawback.

In the present study a group of 115 patients in which all stages were represented was studied (see table 7.2) no correlation between the stages of the disease and ARn was found. The findings of Habib et al (45) appear to be in contrast, however they studied only 13 patients.

Table 7.2 Nuclear androgen receptor (ARn) content of prostatic biopsies according to the stage of the disease. ARn are given as mean ± SEM, n indicates the number of observations.

		ARn	
stage (TNM)	n	fmol/mg protein	fmol/mg DNA
T1-3 NoMo	33	29 ± 4	50 ± 8
T1-3 N+Mo	26	45 ± 15	58 ± 10
T1-3 NxM+	65	43 ± 4	71 ± 8

7.5 Heterogeneity of prostate cancer

There is evidence that, at the time of diagnosis, most human neoplasms are not composed of cells of equal biological behavior. Primary tumors consist of subpopulations of cells with widely different phenotypic characteristics. Cells obtained from individual tumors have been shown to differ with respect to their antigenic properties, growth rate, presence and content of hormone receptors and response to cytotoxic drugs. Heterogeneity is defined "as consisting of, or composed of dissimilar elements or ingredients not having a uniform quality throughout" (48).

Moreover prostatic cancer is usually a most heterogeneous tumor often with areas of varying differentiation intermingled (49). It is increasingly apparent that there is great biological variation even among the histologically similar cells that comprise an individual tumor. The process of biological change in proliferating tumors results in tumor cell heterogeneity and it is this heterogenity that is probably responsible for the varied presentation of prostatic cancer and for variation in clinical response to therapy. The marked heterogeneity of the tumor cell population has been manifested in some individual patients by the appearance of widely spread bony metastases despite shrinkage of the primary tumor and normalisation of prostatic acid phosphatase serum levels values by hormonal manipulation. Further evidence for heterogeneity of prostate cancer has been established by the extensive work of Isaacs and Coffey on the Dunning R-3327 tumor (50). The Dunning R-3327 rat prostatic adenocarcinoma is an appropriate model to study, since it resembles the many variations observed in human prostate cancer. The numerous transplantable solid tumor lines that have spontaneously developed offer a wide range of morphology, growth rate, hormone sensitivity, and aggressiveness (50-52). It can be expected that hormone sensitive and insensitive cells are resident within the same tumor, and it is reasonable to assume that a receptor-rich tumor is dominated by hormone sensitive cells and a receptor poor tumor primarily by hormone insensitive cells. In the present investigation a considerable variation in nuclear

androgen receptor content of multiple biopsies in the same prostatic carcinoma was found (Chapter 4). We attribute these findings to the extensive heterogeneity of the tissue, and not to the amount of tissue used or the validity of the assay. This is illustrated by results obtained, when this method was used for the transplantable human prostatic cancer model PC-82. The intra tissue coefficient of variation for the ARn assay in this supposedly homogeneous tumor was found to be approximately 20% (53). Except for the study of Gorelic et al (54) most studies on the value of the estimation of ARn receptors to day do not mention heterogeneity of receptor distribution over the tissue. They used larger amounts of tissue and only one estimation was done of the whole tissue pool. It is questionable whether the mean receptor content of the primary tumor reflects the true receptor content of the whole tumor load of the patient, especially taking into account the different values of androgen binding found for the primary and its associated metastatic tissue (Chapter 5). In mouse mammary tumors containing mixtures of hormone dependent and autonomous cells (55) it was found that the growth behaviour of heterogeneous tumor grafts that contained more than 10% autonomous cells was essentially determined by these cells. It is questionable whether differences e.g. between 10 and 20% hormone independent cells in an already heterogeneous tumor can be detected on the basis of a receptor assay. The growth rate of these autonomous cells will most likely determine the prognosis and survival of the patient.

7.6 Other methods to determine hormone responsiveness

7.6.1 Methods based on androgen receptor

Present biochemical methods for receptor estimation do not allow us to estimate the relative distribution nor the percentage of hormone dependent and independent cells. Other approaches which would allow determination of the AR-status of individual cells are needed. In this respect, antisteroid antibodies (56) and histochemical methods (57,58) have been used in attempts to establish receptor status. The loss of affinity for the receptor of fluorescent androgen derivatives (59,60) makes that histochemical assays based on these derivatives are not appropriate for detection of androgen receptors in the prostate. Autoradiography has been a useful method in the possible measurement of androgen dependence of human prostatic cancer cell lines (60). In two prostatic adenocarcinoma cell lines Du-145 and PC-3 (61), Du-145 known to be hormone responsive and PC-3 known to be hormone independent, uptake and retention of 3H-R-1881 were only observed and solely confined to the nuclei of the Du-145 cells. With this method it is possible to detect the presence of receptors in different cells in a tissue, and to compare the distribution of receptors to the grade of a tumor. Moreover, it is possible to measure only cancer cells and not other cells such as hyperplastic and "normal" cells, providing additional correlation of structure and function. Peters et al (63) demonstrated the heterogeneity of receptor distribution between cell types of the prostate and illustrated the

improved resolution of this technique over that using whole tissue homogenates.
Androgen receptors have been recently cloned (64,65,66) and major progress will be gained once monoclonal antibodies have been developed.

7.6.2 Non receptor methods

- Ploidy

Tavares et al (67) reported a correlation between ploidy and survival and response to endocrine therapy in 35 patients with prostatic carcinoma. These observations have been confirmed and extended (68,69) demonstrating a correlation between tumor histologic grade and tumor ploidy as determined by cytophotometry. In two studies (70,71) with the R-3327-G rat prostatic adenocarcinoma, the potential usefulness of flow cytometry in measuring response to endocrine therapy was demonstrated.

- Tissue androgen levels

The tissue level of DHT, the major active intracellular androgen, was determined in order to find out whether this level changed in various physiological or pathological states. Geller et al (72) and Belis et al (73) noted that DHT levels lower than 2 mg/g of prostatic cancer tissue were associated with failure to respond to hormonal therapy.
Brendler et al (74) measured DHT content in needle biopsy specimens of stage D2 prostatic cancer taken just prior to initiation of androgen withdrawal, and correlated these

levels with duration of response to treatment. They found no significant difference in the prostatic DHT content of those patients who responded poorly versus those patients who had a good response.

Androgen receptors are assayed in terms of their steroid binding activity, and not in terms of a biological activity. For this reason Brendler et al (29) measured multiple biochemical parameters. Six enzyme activities which might reflect androgen sensitivity; 5α-reductase, 17β-hydroxysteroid oxidoreductase, 3α(β)-hydroxysteroid oxidoreductase and 3 hydrolytic enzymes, i.e. acid phosphatase, alkaline phosphatase and lactate dehydrogenase were measured in prostatic needle biopsies. An index based on multiple relative enzymatic activities was derived empirically which separated the responders and non-responders better than any single variable alone and with less overlap than ARn receptor content did. By analogy with ARn, the enzymes used in such calculations may also be distributed inhomogeneously over the tissue. Clearly this would then influence the results.

- Plasma androgen

Measurement of plasma testosterone before hormonal treatment may predict the probability of a response to androgen deprivation therapy (75,76,77). It is doubtful whether benefit should be expected from treatment aimed at reducing testosterone levels in patients with serum testosterone levels the range of normal women or hypogonadal men.

Isaacs et al (52) concluded in an animal tumor model, the Dunning R-3327-H adenocarcinoma, that progression after

castration is dependent upon the heterogeneity of the H-tumor, or the continuous growth of the androgen independent cells. If this would be equally applicable to human prostate cancer then patients with low serum testosterone levels might already have suppressed their androgen sensitive tumor part prior to the moment they are diagnosed with stage D2 carcinoma of the prostate, and cells are selected by hormonal deprivation. At that moment they may already progress because of the continuous growth of their androgen independent tumor cell population. In this study (Chapter 6) all 7 patients with pretreatment serum testosterone levels lower than 8 nmol/l progressed within 12 months. Other studies (75,76) have yielded similar results. This might indicate that there is a critical level of serum testosterone, meaning that patients with a testosterone level in this region might expect less of hormonal treatment than patients with higher levels, and probably should be treated with other forms of therapy.

7.7 Conclusions

From the results presented in this thesis supplemented with data from the literature, we have concluded that:

- the measurement of ARn content in multiple biopsies has no value in predicting time to progression in stage D2 patients after orchiectomy.
- tumor cell heterogeneity and contamination by nonmalignant prostatic tissue may account for the difficulties in using receptor assays on homogenates of large tissue samples to

predict duration of response of prostatic cancer to endocrine manipulation.

- further progress in developing methods to predict response on hormonal manipulation will only be made when the tissue heterogeneity can be quantified and is taken into account.
- pretreatment testosterone levels might predict time to progression after androgen withdrawal in patients with stage D2 prostatic carcinoma.

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SUMMARY

Target organs for steroid hormones respond to the hormonal stimulus with the aid of specialized proteins termed steroid receptors.

In human breast cancer the presence and concentration of estrogen and progestin receptors is of prognostic significance. Tumors with high receptor levels are more likely to respond favourably to endocrine therapy than tumors with lower levels or without receptors. Because prostatic cancer is also a potentially hormone sensitive tumor, androgen receptors have been assumed to play a role similar to that of the female sex steroid receptors in breast cancer. The aims of the present investigation as described in Chapter 1 were:

- to study the level of nuclear androgen receptors in biopsy specimens of normal, hyperplastic and malignant human prostatic tissue, and
- to evaluate the existence of a possible relationship between the nuclear androgen receptor level and the duration of response after androgen ablative therapy.

In Chapter 2 the discovery of the endocrine dependency of the prostate the rationale for and different forms of endocrine manipulation of prostatic cancer are discussed. After almost 50 years hormonal therapy aimed at androgen ablation remains the first choice of treatment in stage D2 prostatic cancer patients and in terms of duration of response there is no better treatment than castration. The current concepts

regarding the mechanism of action of androgens and the intracellular localization of androgen receptors are also reviewed in Chapter 2. The timing of androgen ablative therapy "early" or "late" is discussed in Section 2.4. and Appendix paper I. Based on the results obtained, early treatment is recommended. A randomized study in patients with stage D1 disease proven by pelvic lymphadenectomy will in the future give a definite answer on the question whether to start hormonal therapy early or late.

Chapter 3 describes the outline of the study, the recruitment of the patients, collection and processing of the specimens. The results of the estimation of the ARn level in biopsy specimens from "normal", hyperplastic and carcinomatous tissue are described in Chapter 4. A considerable variation in ARn content within tumors of individual patients was found, indicating that ARn are not uniformly distributed over prostatic tissue. Prostatic biopsies which were classified as containing normal prostatic tissue had significantly lower ARn values than pathological prostatic tissue. No significant differences were found between hyperplastic and carcinomatous prostatic tissue.

In Chapter 5 it is shown that the ARn content in needle biopsies of prostatic tissue of patients with stage D2 disease was of no value for the prediction of time to progression after orchiectomy. Pretreatment plasma testosterone concentration has been suggested to be predictive for duration of response after androgen withdrawal. Chapter 6 describes the correlation between pretreatment plasma testost-

erone and time to progression after orchiectomy.

In Chapter 7 a general discussion is presented. It is postulated that tumor cell heterogeneity and contamination by nonmalignant prostatic tissue may account for the difficulties encountered when homogenate based receptor assays are used in attempts to predict the duration of response of prostatic cancer to hormonal treatment. Further progress in this field might only be expected from methods which take this heterogeneity into account.

SAMENVATTING

Doelwitorganen voor steroid hormonen hebben specifieke proteinen, welke steroidreceptoren heten, nodig om te kunnen reageren op een hormonale stimulus.

Bij patienten met borstkanker is de aanwezigheid en de concentratie van oestrogeen- en progestageenreceptoren van voorspellende waarde gebleken. Tumoren met een hoog receptorgehalte hebben een betere kans om goed te reageren op hormonale therapie dan tumoren met een laag receptorgehalte of geen receptoren. Omdat prostaatkanker ook potentieel een hormoongevoelige tumor is, werd er verondersteld dat androgeenreceptoren een gelijke rol zouden kunnen spelen als de receptoren voor vrouwelijke geslachtshormonen bij borstkanker.

De doelstellingen van het huidige onderzoek beschreven in hoofdstuk I waren het gehalte van de nucleaire androgeen receptoren in biopsiemateriaal van normaal, hyperplastisch en maligne humaan prostaatweefsel te meten en het bestaan van een mogelijke relatie tussen het gehalte van deze androgeenreceptoren en de duur van respons na androgeen ablatieve therapie te onderzoeken.

In hoofdstuk II, wordt de ontdekking van de endocrine afhankelijkheid van de prostaat, de ratio en de verschillende vormen van endocriene therapie bij prostaatkanker besproken. Na bijna 50 jaar, blijft hormonale behandeling welke gericht is op het onttrekken van androgenen, de behandeling van

eerste keuze bij patienten met een gemetastaseerd prostaatcarcinoom en tot heden is er geen betere therapie dan de orchiedectomie wanneer het de duur van respons betreft. De huidige opvattingen over het werkingsmechanisme van androgenen en de intracellulaire werking van androgeenreceptoren worden besproken. Het tijdstip voor androgeen ablatieve therapie "vroeg" of "laat" wordt ter discussie gesteld in paragraaf 2.4 en appendix paper I. Gebaseerd op de verkregen resultaten wordt vroege behandeling aanbevolen.
Slechts een gerandomiseerde studie bij patienten, die na pelvine klierdissectie in stadium N+ (positieve regionale klieren) verkeren zal in de toekomst de vraag definitief beantwoorden of "vroege" of "late" hormonale behandeling de voorkeur verdient.
In hoofdstuk III wordt de opzet van de studie, het verzamelen van patienten en materiaal alsook de bewerking van het weefsel beschreven. De resultaten van de meting van androgeen kernreceptoren (ARn) in biopsie materiaal van normaal, hyperplastisch en maligne weefsel worden besproken in hoofdstuk IV. Een aanzienlijke variatie in ARn gehalte in tumorweefsel van individuele patienten werd gevonden, hetgeen duidt op een niet uniforme verdeling van de receptor over het prostaatweefsel. Prostaatbiopten die voornamelijk normaal prostaatweefsel bevatten hadden een significant lager ARn gehalte dan pathologisch prostaatweefsel. Tussen hyperplastisch en maligne prostaatweefsel werd geen significant verschil gevonden. In hoofdstuk V wordt aangetoond dat het ARn gehalte van naaldbiopten van prostaatweefsel van

patienten met stadium M+ geen voorspellende waarde had voor de duur van respons na orchiedectomie.

De serum testosteronconcentratie voor behandeling wordt verondersteld een prognostische waarde te hebben voor de duur van respons na het onttrekken van androgenen. In hoofdstuk VI wordt de correlatie tussen de testosteroncon-centratie in serum en tijd tot progressie na orchiedectomie beschreven.

In hoofdstuk VII wordt een algemene discussie gepresenteerd waarin gepostuleerd wordt, dat waarschijnlijk de heterogeni-teit van de tumorcellen en de aanwezigheid van niet maligne prostaatweefsel verantwoordelijk zijn voor de moeilijkheden welke ondervonden worden, wanneer weefselhomogenaten voor receptor bepalingen worden gebruikt, om bij patienten met prostaatkanker duur van respons te voorspellen na hormonale behandeling, en dat men alleen vooruitgang op dit gebied mag verwachten van methoden waarbij deze heterogeniteit in acht genomen wordt.

LIST OF ABBREVIATIONS

A	Androstenedione
ACTH	Adrenocorticotrophic hormone
Alb	Albumin
AR	Androgen receptor
5α-R	5-alpha reductase
BPH	Benign prostatic hyperplasia
CRF	Corticotrophin releasing hormone
DHT	5α-Dihydrotestosterone
DNA	deoxyribonucleic acid
EORTC	European organization for research on treatment of cancer
LH	Luteinizing hormone
LHRH	Luteinizing hormone-releasing hormone
M+	Metastatic disease
NPCP	National prostatic cancer project
PIF	Prolactin inhibiting factor
R*	Activated receptor
RNA	Ribonucleic acid
mRNA	Messenger ribonucleic acid
SHBG	Sex hormone-binding globulin
TNM	Tumor node metastasis
TUR	Transurethral resection

APPENDIX PAPER I

EARLY ORCHIECTOMY FOR PATIENTS WITH STAGE D1 PROSTATIC CARCINOMA

OGJM van Aubel, WJ Hoekstra and FH Schröder

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EARLY ORCHIECTOMY FOR PATIENTS WITH STAGE D1 PROSTATIC CARCINOMA

OLAV G. J. M. VAN AUBEL, WYTZE J. HOEKSTRA AND FRITZ H. SCHRÖDER
From the Department of Urology, Erasmus University Rotterdam, Rotterdam, The Netherlands

ABSTRACT

Staging lymphadenectomy revealed stage D1 disease in 30 of 94 patients with clinically localized prostatic carcinoma. Early orchiectomy resulted in a 46 per cent treatment failure rate after 45 months and established local disease control in almost all patients. The interval to treatment failure in this group compares favorably to the progression rate in patients treated with other modalities.

In 1941 Huggins and Hodges reported that patients with metastatic carcinoma of the prostate improved following bilateral orchiectomy or the administration of estrogens. Since that time therapy aimed at suppression of androgenic stimulation of the prostate became widely used. In the late 1940s and early 1950s hormonal therapy was used for almost every stage of prostatic carcinoma. Today, hormonal therapy is accepted without question for patients with stage D2 symptomatic disease. For patients with stage D1 disease, when the tumor has spread to the pelvic lymph nodes, controversy exists about the best method of treatment. Several reports showed that nodal involvement is a poor prognostic sign irrespective of the given treatment.[1-4] However, the early administration of endocrine therapy seems to enlighten the grave outlook for these patients.[5] We believe that patients with stage D1 cancer have systemic disease and, therefore, must be treated accordingly. In our study an attempt is made to evaluate the impact of staging lymphadenectomy and early orchiectomy in patients with stage D1 prostatic carcinoma.

MATERIALS AND METHODS

From 1977 to 1983, 94 patients with clinically localized prostatic cancer underwent pelvic lymphadenectomy. Patient age ranged from 52 to 72 years, with a mean of 64 years. Followup ranged from 9 to 73 months, with a mean of 34 months. Radical prostatectomy was performed in 64 patients with histologically negative nodes. The technique of lymphadenectomy depended on the presence and extent of nodal metastases. If no gross macroscopic nodes were encountered the dissection included the common iliac, external iliac and obturator nodes. When gross disease was found only some nodes were removed for frozen section and, if positive, the procedure was terminated. Lymph node involvement was found in 30 patients. Early hormonal treatment consisted of bilateral orchiectomy in 28 patients during the same operation and in 2 within 1 month after lymph node dissection. Followup consisted of physical examination, and determination of serum alkaline and acid phosphatase levels every 3 months. Bone scans and chest x-rays were performed at 6 to 12-month intervals. The prostatic volume was determined at regular intervals by transrectal ultrasonography.

Progression of disease was defined as the appearance of distant metastases on chest x-rays or bone scans, elevation of previously normal acid phosphatase levels on 2 consecutive followup examinations, appearance of biopsy proved soft tissue metastases and a 25 per cent or more increase in local tumor volume measured by transrectal ultrasonography. The curve representing time to treatment failure was calculated by the Kaplan-Meier method.[6] Probability of progression ± 2 standard errors for a given time was calculated according to the method of Greenwood.

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RESULTS

Table 1 demonstrates the distribution of the 30 patients according to the tumor, nodes and metastasis grading system, and the N category as determined by pelvic lymphadenectomy. The majority of the patients had a poorly differentiated tumor and positive nodes beyond the obturator nodes. Progression occurred in 7 patients 14 to 45 months after lymph node dissection and subcapsular orchiectomy. Of these 7 patients 5 have died. The Kaplan-Meier curve for interval to treatment failure ± 2 standard errors is demonstrated in the figure. The interval from lymph node dissection and subcapsular orchiectomy to death ranged from 25 to 73 months, while that from progression to death ranged from 11 to 25 months. Another patient died of a myocardial infarction without evidence of progression of the disease.

DISCUSSION

Hormonal therapy is preferred as the initial treatment in patients with stage D prostatic carcinoma. However, controversy exists regarding early or late treatment (when the patient becomes symptomatic). The studies of Blackard[7] and Lepor[8] and their associates showed that the timing of hormonal therapy had no impact on over-all survival.

In patients with clinically localized prostatic adenocarcinoma and positive nodes a radical operation (lymph node dissection and radical prostatectomy) or external beam radiotherapy will provide evidence of treatment failure in 40 per cent within 2 years.[4,9-11] Some investigators claim that minimal node involvement is not as poor a prognostic sign as originally believed,[3,5,12,13] while others could not confirm such a relationship between the volume of positive nodes and prognosis.[4,14] From many studies it is clear that the survival rate of patients with positive nodes at pelvic exploration is significantly worse than that of patients with negative nodes. Once pelvic nodes are involved the chance of having other undetected metastases is high. Some studies reported rapid progression of the disease in patients with stage D1 prostatic carcinoma treated by a radical operation, radiation and/or delayed hormonal treatment.[2-5,9]

Pelvic lymphadenectomy combined with radiotherapy or a radical operation provides a median interval to progression of 15.8 to 36 months.[2-4,9] In patients with stage D1 carcinoma who are treated with delayed endocrine therapy the median interval to treatment failure is 12 months.[6,15] Early endocrine therapy after radical prostatectomy in these patients prolongs the interval to treatment failures.[2,5]

In our study early hormonal treatment resulted in a 46 per

APPENDIX PAPER II

NUCLEAR ANDROGEN RECEPTORS IN HISTOLOGICALLY NORMAL, HYPERPLASTIC AND CANCEROUS HUMAN PROSTATIC TISSUE

OGJM van Aubel, J Bolt-de Vries, MA Blankenstein, FWJ ten Kate and FH Schröder

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NUCLEAR ANDROGEN RECEPTORS IN HISTOLOGICALLY NORMAL, HYPERPLASTIC AND CANCEROUS HUMAN PROSTATIC TISSUE

O.G.J.M.van Aubel, J. Bolt-de Vries, M.A.Blankenstein*, F.W.J. ten Kate**, F.H.Schröder
Departments of Urology and Pathology**, Medical Faculty, Erasmus University Rotterdam, Rotterdam, The Netherlands.
*Present address: Department of Biochemistry, Dr.Daniel den Hoed Cancer Center, Rotterdam, The Netherlands

INTRODUCTION

In patients with breast cancer, the estimation of the estrogen receptor content is an accepted parameter for selection of treatment and prognosis (1). By analogy with breast cancer, the androgen receptor content of prostatic tumors has been investigated as a means of predicting the response of prostatic carcinoma to endocrine therapy. Trachtenberg and Walsh (2) and Ghanadian et al (3), both estimating cytoplasmic as well as nuclear androgen receptors, found that nuclear androgen receptor content was related to duration of response (2, 3) and survival (2) following hormonal therapy in advanced prostatic cancer. The clinical value of the estimation of androgen receptors is still a matter of debate (4).

Patients with advanced prostatic carcinoma are not eligible for open surgical procedures. Therefore biopsy of the prostate is the only acceptable alternative to provide small amounts of tissue (± 25 mg per biopsy). A method for the estimation of nuclear androgen receptors has been published (5), and this method has recently been made applicable to biopsy specimens (6). The aims of the present investigation were to find out, using biopsies as small as 25 mg, if there are differences in the nuclear androgen receptor content of normal and pathologic human prostatic tissue, and to start a prospective study on the clinical value of this nuclear receptor estimation in biopsy specimens of prostatic carcinoma.

MATERIALS AND METHODS

Tissue Prostatic tissue was obtained from two different group of patients by means of 2-4 perineal prostatic biopsies. One group of patients (n=83) underwent a diagnostic biopsy of their prostates. The other group (n=60) was biopsied prior to undergoing transurethral resection of their prostates for benign hyperplasia. Samples were immediately frozen in liquid nitrogen and stored at -80°C. A frozen section was made of each single biopsy for histological diagnosis. If the amount tissue exceeded 50 mg, more than one receptor assay was done. Histological analysis of these biopsies showed that 10 out of 322 were normal, 140 showed BPH and 172 showed carcinoma. Tissue showing severe signs of infection was excluded from this study, as were biopsies showing less than 50% carcinomatous tissue. In the BPH group a rough estimate was made of the percentage of epithelium in the specimen.

Assay of nuclear androgen receptors. The content of androgen receptors was estimated by an assay (5, 6) which involves extraction of nuclear pellets with a heparin containing buffer, exchange labelling of the nuclear extracts with 10^{-8} mol/liter ^{3}H-R1881 at 10°C in the presence of a 500-fold triamcinolone acetonide, and quantification of the receptors with protamine sulphate precipitation. Correction for aspecific binding was made by a parallel incubation in the presence of a 200-fold excess unlabelled R1881.

Other procedures. The protein concentration of nuclear extracts was determined according to Peterson (7). DNA was estimated in nuclear pellets with the method of Hinegardner (8).

Statistical evaluation. The significance of differences was tested by Wilcoxon's Test. Differences were considered to be statistically significant when p-values less than 0.05 were obtained. The existence of correlations was tested with Spearman's rank correlation test.

RESULTS

Androgen receptors were detected in the nuclear extracts (ARn) of 103 out of 115 samples (90%) from prostatic carcinoma, in 66 out of 70 samples (94%) from hyperplastic prostates and in 4 out of 6 samples from tissue which was histologically classified normal (NP), but obtained from benign hyperplastic (BPH) or malignant prostates (PC). The results of the measurement of androgen receptors in the samples are shown in Figure 1.

Biopsies from BPH and PC had comparable contents of ARn. The nuclear androgen receptor content of biopsies which were classified normal was significantly lower than that of BPH and PC biopsies.

In Figure 2 the content of ARn for PC biopsies of different histological grade is given. The ARn content of G1 prostatic carcinomas appeared to be significantly lower than that of tumors of the other histological grades (Figure 2).

For hyperplastic prostates, a significant correlation (R_S= 0.36, $p < 0.001$) was found between the estimated percentage of epithelium and the ARn level. The nuclear androgen receptor levels of the biopsies of BPH and PC were independent on the age of the patients.

When more than one receptor estimation of the same tumor was performed, a considerable variation in receptor content was found. For patients for which three or more samples were available, a coefficient of variation of 57 ± 28% (mean ± s.d.), ranging from 30-109% was found (data not shown).

No correlation was found between the ARn content of prostatic biopsies (PC) expressed as fmol per mg DNA and the DNA content of the nuclear pellet (Figure 3). There was a good correlation between the ARn content in PC expressed as fmol per mg protein and as fmol per mg DNA (Figure 4).

DISCUSSION

The values observed in the present study for the androgen receptor content of prostatic tissue, BPH and PC are comparable with the findings of Bruchovsky et al (9) and Barrack et al (10). When we recalculated our data using a DNA content of 27 pg/nucleus (9), the range of the observed numbers of receptor sites was 500-4000 sites/nucleus, which is well in agreement with the published data (9). By contrast, our results are not in agreement with those of Ghanadian et al.(3) and Shain et al.(11). Differences in methodology may be the cause of this discrepancy.

The normal tissue in this study was obtained from patients of the same age as the patients with pathologic prostates. Thus the differences in receptor level measured in normal tissue and pathologic tissue cannot be explained by age-dependent physiological changes, which appear to exist in other androgen target tissues (12). In the BPH group a correlation was found between the percentage of epithelium and the nuclear androgen receptor content. This result suggests, that the ARn level of epithelium is higher than that of the stromal compartment. Patients with tumors of higher differentiation grades are known to have better prognosis than patients whose tumor is less well differentiated. Thus we expected to find a correlation between the histological grade of the tumor and its androgen receptor content. In contrast to our expectation, however, G1 tumors had a lower ARn content than the less well differentiated tumors. At present, the physiological significance of this observation remains unclear.

In this investigation a considerable variation in nuclear androgen receptor content of multiple biopsies in the same prostatic carcinoma was found. This suggests that androgen receptors are distributed inhomogenously over prostatic tissue. Alternative ly prostatic tumors may be composed of mixtures of preexisting clones of tumor cells with different amounts of nuclear androgen receptors. The extent of variation of ARn levels and the variable number of biopsies which can be obtained of the primary tumor, rais the question whether the mean of these values reflects the true receptor content of the whole tumor load of a patient or whether the hormone dependence of the tumor is determined by the samples with the highest or lowest receptor content respectively. The results of Sluyser et al.(13) with mammary tumors are noteworthy in this respect.

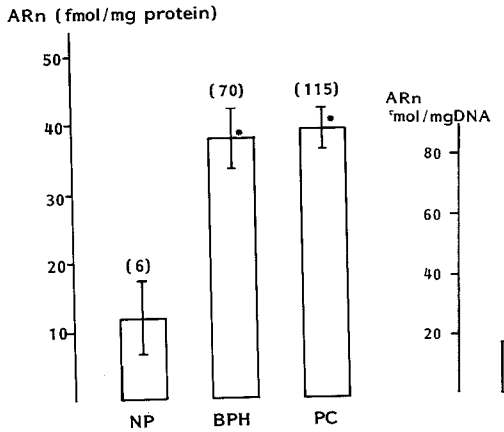

Figure 1. Nuclear androgen receptors (ARn) in human prostatic biopsies. Results are expressed as means ± S.E.M. Number of estimations are mentioned in the parenthesis. *Significantly different from normal prostate (NP) group.

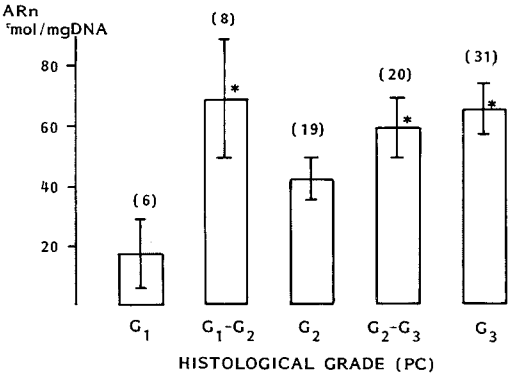

Figure 2. Nuclear androgen receptors (ARn) in biopsies of prostatic carcinoma of different histological grades. Results are expressed as means ± S.E.M. Number of estimations are mentioned in the parenthesis. *Significantly different from G1 group ($p<0.05$).

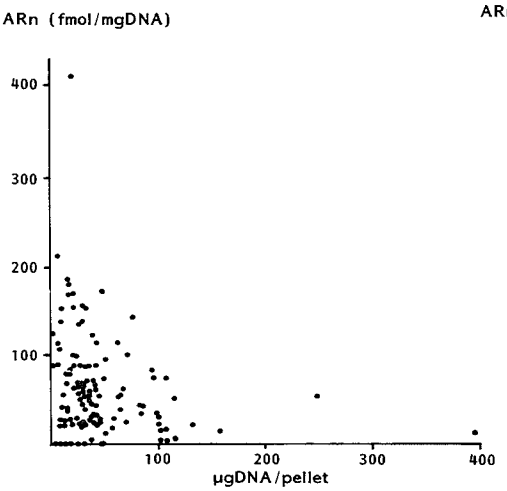

Figure 3. Lack of correlation between the nuclear androgen receptor (ARn) content of prostatic biopsies (PC) and DNA content of the nuclear pellet (R_s=0.11, n=140, $p>0.10$)

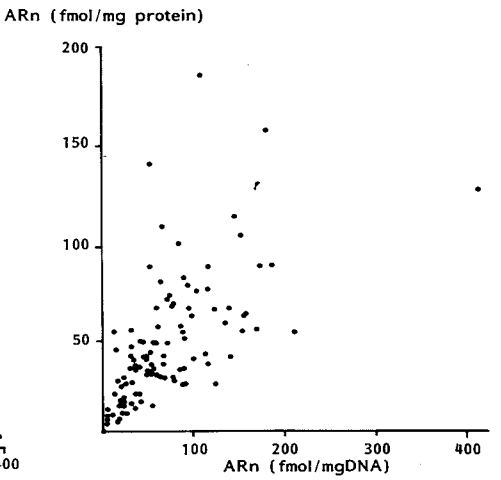

Figure 4. Correlation between nuclear androgen receptors (ARn) in PC expressed as fmol per mg protein and as fmol per mg DNA (R_s=0.70, n=140, $p<0.001$).

They found that the growth behavior of heterogeneous mammary tumor grafts that contained more than 10% autonomous (hormone-independent) cells, was essentially determined by these cells. It is questionable whether small differences e.g. between 10 and 20% hormone-independent cells can be detected on the basis of a receptor assay. The results obtained by Ghanadian et al. (3) with tissue from transurethral resection and Trachtenberg et al. (2) with large biopsies suggest that ARn can be used as prognostic indicator.

A prospective study has been started to evaluate whether single needle biopsies can also be used to evaluate the prognosis. At present 82 patients have entered this study.

CONCLUSIONS

* Nuclear androgen receptor (ARn) content of normal prostatic tissue is significantly lower than that of pathologic tissue.
* There is no significant difference between the ARn content of hyperplastic and carcinomatous prostatic tissue.
* The observed correlation between ARn content and the percentage epithelium in BPH, suggests that epithelium contains more ARn than the stromal compartment.
* The ARn concentration is independent on the DNA content of the nuclear pellet, the age of the patients and dependent on the grade of the tumor (PC).
* ARn levels can be expressed with respect to either the content of protein in the nuclear extract or the amount of DNA in the nuclear pellet.
* In some tumors a severe inhomogeneity in the ARn concentration was found.

The question whether the behavior of the tumor is predicted adequately by the ARn content of a single small biopsy, by the mean receptor level of a number of such biopsies, or by the region with the highest or lowest receptor content, remains to be answered.

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NAWOORD

Op deze plaats dienen eerst de patiënten genoemd te worden die er in toestemden dat meer prostaatbiopten van hen verzameld werden dan gebruikelijk is. Zonder hun medewerking was dit onderzoek niet mogelijk geweest.

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CURRICULUM VITAE

De schrijver van dit proefschrift behaalde in 1969 zijn diploma gymnasium β te Zeist (Katwijk de Breul). Vanaf 1970 studeerde hij Geneeskunde aan de Rijksuniversiteit te Leiden en behaalde het artsexamen in 1976. Van 1976 - 1977 was hij research assistent op de afdeling urologie van het Academisch ziekenhuis Leiden, hoofd Prof. Dr. P.J. Donker met als onderwerp spierfysiologie van de musculus sfincter externus urethrae. De chirurgische vooropleiding ten behoeve van de specialisatie urologie werd gevolgd op de afdeling heelkunde St. Jozef Ziekenhuis te Deventer, opleider Dr. S.J. Rinsma (1977 - 1980). De opleiding tot uroloog werd gestart in 1980 op de afdeling urologie van het Academisch Ziekenhuis te Rotterdam, hoofd Prof. Dr. F.H. Schröder en op de afdeling kinderurologie van het Sofia kinderziekenhuis te Rotterdam, hoofd Prof. Dr. R.J. Scholtmeyer. Op 15 juli 1984 volgde, na afronding van het perifeer jaar op de afdeling Urologie van het St. Franciscus Gasthuis te Rotterdam, hoofd Dr. S.I. Miranda, de inschrijving in het specialistenregister.
Vanaf 1984 is hij werkzaam als uroloog in het Franciscus ziekenhuis te Roosendaal en sinds 1986 ook in het Lievensberg Ziekenhuis te Bergen op Zoom in associatie met J.W.M.H. Plasman en H.C. Pull.