Urol Res (1989) 17:99–102

Urological Research

Circulating testosterone, prostatic nuclear androgen receptor and time to progression in patients with metastatic disease of the prostate treated by orchiectomy

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Accepted: October 1, 1988

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 [4]. Recently pre-treatment plasma testosterone has been suggested to be predictive in this respect [5]. Therefore, pre-treatment plasma testosterone (T) and sex hormone binding globulin (SHBG) levels were studied in 31 patients aged 72 ± 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 ± 41 fmol/mg protein; range 0–169). Plasma T levels (mean: 13.7 ± 6.1 nmol/l) varied widely (range: 2.4–25.4), as did plasma SHBG (32.5 ± 19.3 nmol/l; range 4.4–78.8), and time to progression (TTP; 14.6 ± 11.2 months; range 1–48). Plasma T was found to be correlated to age ($Rs = 0.537$; $P < 0.01$) and TTP ($Rs = 0.4495$; $P < 0.02$). Tissue ARn and plasma SHBG did not correlate to any of the parameters studied.

Key words: Androgen receptor – Testosterone – SHBG – Prostate cancer – Needle Biopsy – Prognosis

Introduction

In 1941, Huggins and Hodges [1, 2] established the scientific basis for the androgen dependence of most prostatic cancers and demonstrated the beneficial effects of orchiectomy or of oral estrogens in most patients with metastatic disease. The degree and the duration of response to androgen deprivation, however, are variable: 10% of patients die within six months, 50 percent of patients have a survival of less than 3 years and only 10% 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 (TN×M1) proven by bone scan. None of these patients had received any orm 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

Part of this paper was presented at the 6th Congress ofthe European Society for Urological Oncology and Endocrinology, May 2–4, 1988, Innsbruck, Austria

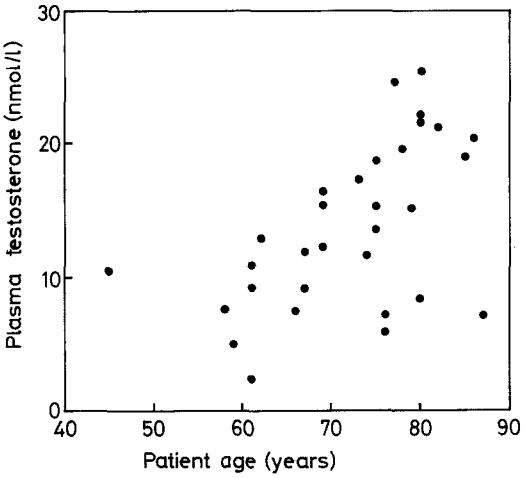

Fig. 1. Pretreatment plasma testosterone versus age in men with advanced prostatic carcinoma (Rs = 0.5371; $P < 0.01$; $n = 31$)

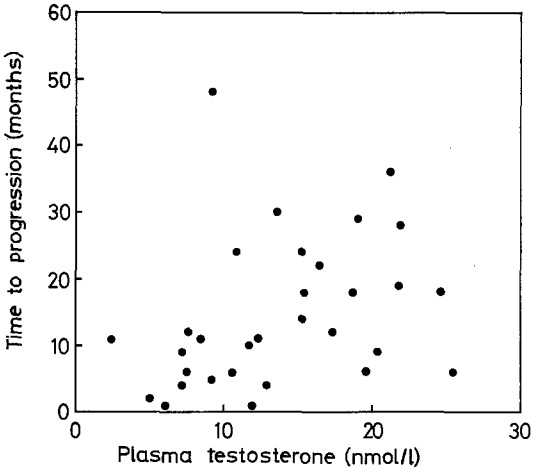

Fig. 2. Time to progression after endocrine treatment versus plasma testosterone (Rs = 0.4495; $P < 0.02$; $n = 31$)

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 were known. Patients were evaluated by follow-up at three-monthly intervals by history, physical examination, routine and specific laboratory examination and at six-monthly intervals by X-ray of the chest and by a bone scan. Progression was defined as appearence of new distant metastases on bone scans, or on X-rays, and where possible were proven by biopsy. An increase in volume of the primary tumor was proven by ultrasound or by 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 involved 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 nonspecific 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 by the method of Hinegardner [9].

Steroid measurements

Plasma testosterone was measured by radioimmunoassay as described previously [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 mehod 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.

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

TTP = time to progression; ARn = nuclear androgen receptor; T = testosterone; SHBG = sex hormone binding globulin; "Free T" = calculated free testosterone concentration

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 ± 41 fmol/mg protein: range (0–169). Plasma T levels (mean: 13,7 ± 6.1 nmol/l) varied widely (range: 2.4–25.4), as did plasma SHBG (33.5 ± 19 nmol/l; range 4–79), and time to progression (TTP; 14.6 ± 11.2 months; range 1–48).

Statistically significant correlations were found between plasma T and age (RS = 0.537; $P < 0.01$),

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

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

Fig. 1, and between plasma T and TTP (Rs = 0.4495; $P<0.02$) Fig. 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 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 disease are aimed at finding a difference in testosterone levels between patients with prostatic cancer and those with benign hyperplasia or those with a normal prostate [15–17]. Other studies have sought to find a relationship between plasma testosterone and stage [18, 19] and grade [18]. In this study we evaluated the relationship between pretreatment 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 agreed 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 to less well defined parameters, response and/or survival, indicated 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 were shown to 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 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 at the beginning of the malignant process, or the serum testosterone was lowered as the patients' general condition deteriorated. In either case patients presenting with low serum testosterone at the time of diagnosis of stage D2 carcinoma of the prostate might be considered to have experienced already a period of androgen withdrawal before diagnosis. These patients have already relapsed, and 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 measurement of plasma testosterone prior to the start of androgen suppressive therapy, in order to obtain an indication of the probable effect of this treatment.

Acknowledgement. We thank Mrs. Merks-v. Bastelaar for typing the manuscript.

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