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Steroid Receptors in Metastatic Carcinoma of the Human Prostate*

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Abstract—*Using a dextran-coated charcoal technique and isoelectric focusing in polyacrylamide gel, metastases from five patients with prostatic carcinoma were analysed for contents of cytosolic androgen, progestin, estrogen and glucocorticoid receptors. Maximum binding capacity* (B_{max}) *and dissociation constant* (K_d) *were calculated from Scatchard plots. In three cases the patients had received endocrine therapy prior to removal of the specimen. Androgen and progestin receptors were measurable in* 4/5 *and* 2/5 *cases, respectively; no specimen contained estrogen receptors. In* 3/5 *cases large amounts of glucocorticoid receptors were detected.*

INTRODUCTION

MEASUREMENT of steroid receptors is of importance in human malignancy since determination of intracellular receptor amounts in tumours of hormone target organs may be of value in predicting the individual response to endocrine therapy. A correlation has been shown between tissue content of estrogen and progestin receptors in disseminated breast cancer and response to hormonal therapy [1]. We have recently reported on an 80% correlation between content of methyltrienolone receptors in needle biopsies from primary prostatic carcinomas and short term response to endocrine therapy [2].

The present study is an investigation of the steroid receptor profiles in metastases from patients with prostatic carcinoma. Some of the patients had been treated with hormones before removal of the metastatic tissue. Measurement of steroid receptor content in tumour tissue may increase our understanding of mechanisms involved in changes of hormonal responsiveness during the course of endocrine therapy.

MATERIALS AND METHODS

Materials

Lymph node metastases from 5 patients suffering from prostatic carcinoma were investigated. One of the metastases was removed from the abdominal wall, one from the retroperitoneal region and three from the left supraclavicular fossa. Parts of each specimen were taken for histological examination. Four of the metastases were of a low degree of differentiation; the fifth cancer (case V) showed a moderate to low degree of differentiation.

Three of the patients had received endocrine therapy either with estramustine phosphate (Estracyt®), 560–840 mg/day, or polyestradiol-phosphate (Estradurin®), 80 mg/month, combined with ethinylestradiol (Etivex®), 150 μg/day, for varying periods of time prior to removal of the specimen. Case V was on treatment with digitoxin, a drug which may interact with estrogen receptors [3]. The mean age of the patients was 67 yr (range 57–76 yr). The choice of treatment was not in any case influenced by the results from the receptor measurements.

Methods

All tissue specimens were cooled on ice and frozen to −70°C within 1–2 hr following the operation. The analyses of cytosolic receptors were performed after storage for 1–2 weeks, a time lag probably not influencing the results of the assays [4]. The methods are described in detail elsewhere [4, 5]. For analysis of androgen receptor content [6, 7-^{3}H] methyltrienolone (17β-hydroxy-17α-methyl-estra-4, 9, 11-trien-3-one, R 1881; specific radioac-

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tivity 55.5 Ci/mmole) [6] was used. Estrogen and progestin receptors were determined using [6, 7-^{3}H] R 2858 (11β-methoxy-17α-ethinyl-estradiol; specific radioactivity 52.0 Ci/mmole) [7] and [6, 7-^{3}H] R 5020 (17α, 21-dimethyl-19-nor-4, 9-pregnadiene-3, 20-dione; specific radioactivity 56.5 Ci/mmole) [8], respectively. Labelled and unlabelled R 1881, R 2858, and R 5020 were generous gifts from Dr. J.-P. Raynaud, Roussel-UCLAF, Romainville, France. [6, 7-^{3}H]-labelled and unlabelled dexamethasone (9α-fluoro-11β, 17α, 21-trihydroxy-16α-methyl-1, 4-pregnadiene-3, 20-dione; specific radioactivity 33.0 Ci/mmole) were used for analysis of glucocorticoid receptors and were purchased from New England Nuclear, Boston, MA and Sigma Chemical Co., St. Louis, MO, U.S.A., respectively.

Multiple incubations with varying amounts of steroid were performed. Calculations of maximum binding capacity (B_{max}) and dissociation constant (K_d) were made from Scatchard plots [9]. The results were corrected according to Chamness and McGuire [10] using an interactive system on a NORD-computer. In all cases B_{max} was related to g of tissue, mg of protein and mg of DNA. Protein was measured according to Lowry [11] as modified by Peterson [12] and DNA according to Burton [13] as modified by Giles and Myers [14].

Determination of estrogen receptor content was also carried out with isoelectric focusing in polyacrylamide gel as described elsewhere [4, 15].

RESULTS

B_{max} values for each case are presented in Table 1. The steroid receptor profiles varied extensively. Four of five specimens contained methyltrienolone-binding sites (range 20–8000 fmole/mg DNA). Progestin receptors were detected in 2/5 cases whereas estrogen receptors were not detectable in any case, either with dextran-coated charcoal technique (0/5) or with isoelectric focusing (0/4). Three out of five specimens contained large amounts of glucocorticoid receptors.

Mean values for dissociation constant (K_d) were 3.1×10^{-10}M ($n=4$) for methyltrienolone, 8.4×10^{-10}M ($n=2$) for R 5020 and 36.0×10^{-10}M ($n=3$) for dexamethasone-binding sites (Table 2).

Relevant clinical data are best reported by a short summary of each case.

Case I

A 69-yr-old man had a moderately well differentiated adenocarcinoma of the prostate diagnosed in 1970. The tumour was well controlled with Estradurin® combined with Etivex® until 1977, when the disease had a marked progress. The patient had severe pains from multiple skeletal metastases. A 100-fold increase in serum acid phosphatase was registered. Lymph node metastases from the left supraclavicular fossa were removed by open surgery in November 1977 and were found to contain large amounts of methyltrienolone and glucocorticoid receptors. No progestin or estrogen receptors could be detected. The therapy was changed to Estracyt® and the patient was rapidly improved. The acid phosphatase level was normalised, Z-ray showed sclerosis of previously osteolytic metastases and the pains were relieved. The patient is still alive and in good condition (June 1978).

Case II

A 64-yr-old man was submitted to the hospital for examination of a high sedimentation rate and skeletal pains. A fairly small poorly differentiated carcinoma of the prostate was found with metastases to the left supraclavicular fossa. The metastasis removed contained only a very small amount of methyltrienolone receptors and no other measureable receptor proteins. The patient was given Estracyt®, but the disease progressed rapidly and the patient died after 2 months.

Case III

A 57-yr-old man had a moderately well differentiated prostatic carcinoma since 1971. The growth of the tumour was initially controlled with Estradurin® combined with Etivex®. Due to progress of the disease, the therapy was changed to Estracyt® in 1976. A short time of remission followed, but in 1977 the disease again had a rapid progress. A biopsy was taken from a large retroperitoneal mass consisting of poorly differentiated adenocarcinoma. The tissue contained no demonstrable steroid receptors. The patient died soon thereafter.

Case IV

A 70-yr-old man had a poorly differentiated prostatic carcinoma diagnosed in January 1977. During external radiation therapy the patient developed lymph node metastases. Following irradiation further therapy with

Table 1. Maximum number of specific binding sites (B_{max} values) for MT, R 5020, R 2858 and dexamethasone in 5 specimens of metastatic prostatic carcinoma

Case No.	Age of patient	Treatment before removal of specimen	Methyltrienolone receptors			R 5020 receptors			R 2858 receptors	Dexamethasone receptors		
			fmole/g tissue (95% confidence limit)	mg protein	mg DNA	fmole/g tissue (95% confidence limit)	mg protein	mg DNA		fmole/g tissue (95% confidence limit)	mg protein	mg DNA
I	69	Estradurin® Etivex®	3910 (3620–4310)	68	674	n.d.	n.d.	n.d.	n.d.‡	13600 (10100–23400)	238	2340
II	64	—	110 (94–155)	3	21	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
III	57	(Estradurin® Etivex®) Estracyt®	n.d.†	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.‡	n.d.	n.d.	n.d.
IV	70	Estracyt®	28500 (21500–45800)	536	8150	1410 (1060–2250)	26	402	n.d.‡	4730 (4250–5400)	89	1350
V	76	—*	12900 (11000–16000)	220	1320	524 (419–734)	9	54	n.d.‡	3910 (3660–4230)	67	401

*Patient treated with digitoxin.
†n.d. = not detectable (slope of Scatchard plot not significantly [$P<0.05$] different from zero).
‡Also analysed with isoelectric focusing in polyacrylamide gel.

Estracyt® was given. Some metastases decreased in size while others increased. In February 1978 one metastasis in the abdominal wall was removed and found to contain large amounts of methyltrienolone receptors as well as glucocorticoid and progestin receptors in considerable amounts. The patient was in an extremely bad condition, had severe pains and the abdomen was full of ascites. A bilateral orchiectomy was performed, but the patient died soon afterwards before any effect of the endocrine manipulations could be evaluated.

Table 2. Dissociation constant $(K_d) \times 10^{-10}$ M ±S.E.M. *for steroid receptors in metastatic prostatic carcinoma*

Case No.	Methyltrienolone	R 5020	Dexamethasone
I	1.1±0.1	—	62.5±12.9
II	2.0±0.5	—	—
III	—	—	—
IV	5.1±0.5	9.4±1.9	28.6±2.4
V	4.1±0.5	7.5±1.1	16.9±1.0
Mean values	3.1	8.4	36.0

Case V

A 76-yr-old man was submitted to the hospital due to anemia, high sedimentation rate and severe skeletal pains. The examination revealed a moderately to poorly differentiated adenocarcinoma of the prostate with metastases to the skeleton and lymph nodes in the left supraclavicular region. The metastatic tissue contained significant amounts of methyltrienolone and glucocorticoid receptors and also detectable levels of progestin receptors. An orchiectomy was performed and within 3 months a 10-fold decrease of the elevated serum acid phosphatase levels was noted, the skeletal pains were totally relieved and the primary tumour could no longer be palpated. Half a year after the castration the patient is still in a good condition with no signs of reactivation of the disease.

DISCUSSION

We have previously demonstrated a good correlation between the methyltrienolone receptor content in prostatic carcinoma and the response to endocrine therapy [2]. In agreement with these results the methyltrienolone "receptor-poor" case II did not respond to treatment with Estracyt® whereas the methyltrienolone "receptor-rich" case V rapidly improved following orchiectomy. Furthermore, the lack of measurable receptors in case III fits well with the unresponsiveness to endocrine therapy that this patient had developed following several years of different hormonal treatments. Likewise, case I had become unresponsive to estrogen treatment after several years. This patient, however, turned out to be "receptor-rich" with regard to methyltrienolone- and glucocorticoid-binding sites and rapidly recovered when receiving an alternative form of endocrine therapy (Estracyt®). Case IV was "receptor-rich" but did no longer respond to Estracyt®; the patient died before effects of alternative forms of endocrine therapy could be evaluated.

These results could be taken as a further support for the suggestion that steroid receptor measurements in prostatic carcinoma may help the clinician to select the optimal therapy (endocrine vs non-endocrine) in the individual case. Patients with "receptor-rich" tumours might be considered for alternative forms of hormonal treatment if the endocrine therapy first chosen is inefficient.

The high methyltrienole receptor levels in cases I, IV and V (674–8150 fmole/mg DNA) contrast to the concentrations previously found in primary prostatic carcinomas (51–318 fmole/mg DNA) [2]. One possible explanation is that the endocrine therapy in cases I and IV by reducing the amount of circulating androgen has created an increased number of unoccupied cytosolic receptor sites. Another contributing factor may be that the metastases contained a higher concentration of cancer cells than the needle biopsy specimens obtained from primary tumours [2].

The rationale for using methyltrienolone receptor levels in prostatic carcinoma to indicate responsiveness to estrogen therapy is that this treatment leads to decreased release of LH from the pituitary gland and, hence, to decreased androgen production from the testes [16]. So far there is no convincing evidence for a direct action of estrogens on the prostatic cell. We have failed to demonstrate estrogen receptors in benign prostatic hyperplasia [4] and the present investigation gives no support for the presence of estrogen receptors in prostatic carcinoma.

Progestin receptors are present in most cases of benign prostatic hyperplasia, but are less common in "normal" prostatic tissue [4, 17]. Progestin receptors were also detected in 2/5 specimens of prostatic carcinoma in the present

investigation. Progestin therapy has been claimed to have a good effect in several cases of prostatic carcinoma [18] and may be considered in cases of progestin "receptor-rich" prostatic tumours.

Measurable quantities of glucocorticoid receptors seem to be absent from normal and hyperplastic prostatic tissue [4]. Three of the five metastatic tumours in the present study, however, contained quite substantial amounts of glucocorticoid receptors. It has been shown that lymphocytes contain receptors for glucocorticoid hormones [19]; however, it is unlikely that the small amount of lymphatic cells in the metastatic specimens (<2%) could account for the high concentration of glucocorticoid receptors. An important question is whether the appearance of glucocorticoid receptors in prostatic tissue runs parallel with tumour responsiveness to glucocorticoid hormones. Treatment with glucocorticoids is sometimes of benefit in disseminated prostatic carcinoma [20]. The mechanism behind this effect has been thought to be suppression of adrenal androgen production, but the present demonstration of glucocorticoid receptors in prostatic carcinoma may suggest an additional direct effect of glucocorticoids on the cancer tissue.

In conclusion, the present investigation indicates that the steroid receptor profile in metastases of prostatic carcinoma varies greatly from patient to patient and supports the contention that receptor concentrations may guide the clinician in selecting the optimal therapy for the individual patient.

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