Estrogen, Progesterone, and Androgen-Binding Sites in Renal Cell Carcinoma

Observations Obtained in Phase II Trial of Flutamide

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A Phase II disease-oriented drug trial using flutamide (4′-nitro-3′trifluoro-methylisobutyranilide) 250 mg by mouth three times a day was undertaken in 28 patients with advanced, bidimensionally measurable renal cell carcinoma. Of 25 adequately treated cases, 1 (4%, 95% confidence limits 0–12%) had a partial remission lasting 9+ months, and 2 had stabilization of disease lasting 6 and 15 months, respectively. Flutamide demonstrated no significant antitumor activity in patients with disseminated renal cell carcinoma. Including patients entered in this study, 62 specimens were evaluated for steroid binding sites using a fluorescent method: 33 of 62 specimens assayed showed no hormone-binding sites, and only 12 cases had androgen binding. Of the 12 of 23 patients receiving flutamide who were biopsied and had an adequate sample for steroid-binding site determination, estrogen binding was demonstrated in 6, androgen binding in 3, and progesterone binding in 4. Since this study did not obtain a sufficient number of cases with androgen-binding positivity, the possible efficacy of flutamide in such cases cannot be excluded.
Cancer 54:477–481, 1984.

RENAL CELL CARCINOMA has been thought to be a hormonally responsive tumor since Matthews and associates[1] in 1947 induced malignant renal tumors in male syrian hamsters by prolonged administration of diethylstilbesterol (DES). Further studies[2] demonstrated that such tumors could be inhibited by prior progestins or testosterone. In 1963 Bloom[3] reported the antitumor effect of orchiectomy on transplanted estrogen-induced tumors in male hamsters and in clinical studies; four responses were noted in 20 patients treated with either dihydroxyprogesterone acetate or testosterone. While some observ-

ers[4,5] initially described a 15% to 35% remission rate in patients with disseminated renal cell carcinoma given progestins, androgens, and/or antiestrogens, the overall response rate to hormonal therapy is generally considered to be 2% to 6%,[6,7] being somewhat higher in males compared with females. In fact, the Renal Carcinoma Study Group[8] noted no advantage of medroxyprogesterone over placebo in inducing regression of metastases in 31 patients with renal cell carcinoma. Megesterol[9] produced a 3.5% partial remission (PR) rate in 85 cases. Tamoxifen[7] produced objective responses in 6.3% of 79 patients. Estramustine phosphate,[10] nafoxidine,[11] and hormones combined with cytotoxic[12] or immunotherapeutic[13] drugs have had similar disappointing results. Despite the insignificant response rates with hormonal manipulation, progestins are considered as primary therapy by some investigators,[14] and, in fact, have been employed adjuvantly.[14,15]

Recent reports indicate the presence of steroid receptors in animal and human hypernephroma cells.[16-17] The presence of androgen receptors in normal male and female mouse kidney was demonstrated by Bullock and Bardin.[18] Likewise, Pertschuk and Brigati[19] using a histochemical technique, demonstrated the presence of androgen-binding sites in 20% of 40 tumor specimens.

Of interest is the fact that antiandrogens have never been used in patients with hypernephroma. Flutamide (4′-nitro-3′trifluoro-methylisobutyranilide) a synthetic,

nonsteroidal, antiandrogen without androgenic, estrogenic, antiestrogenic, corticoid, progestational, antiprogestational, or antigonadeotrophic activity[20] has demonstrated significant antitumor activity in patients with prostatic carcinoma.[21] Thus, a Phase II disease-oriented drug trial was undertaken at Memorial Sloan-Kettering Cancer Center in patients with advanced, bidimensionally measurable, renal cell carcinoma attempting to correlate hormonal response to flutamide with the presence or absence of androgen-binding sites.

Materials and Methods

All 28 patients had a complete history, physical examination, pathologic confirmation of renal cell carcinoma by the Department of Pathology, Memorial Hospital, automated blood cell and platelet counts, 12-channel biochemical screening profile, chest roentgenograms, and in the latter half of the study, luteinizing hormone and testosterone assays. Where indicated, radionuclide liver and bone scans, intravenous pyelograms or computerized transaxial tomograms (CT scans) of the abdomen were obtained. Steroid-binding sites were qualitatively assayed on material obtained at the time of aspiration biopsy or surgery by the fluorescent method of Pertschuk and associates.[22] Entry criteria for this study were strict and required the presence of bidimensionally measurable disease such as peripheral pulmonary lesions, subcutaneous lesions, malignant hepatomegaly evaluable by physical examination and radionuclide and/or CT scans, and biopsy-proven intraabdominal or pelvic tumor measurable by CT scan. Other criteria for patient entry included Karnofsky performance status (PS) $\geq$ 50%, life expectancy $\geq$ 2 months, normal hematologic values, no hormonal therapy within 8 weeks prior to flutamide, and informed consent.

Perpendicular diameters of each accessible lesion were measurable by two investigators. All radiologic studies were evaluated independently (R.C.W.). Follow-up physical examination, biochemical and hematologic studies, and chest x-rays were performed every 3 weeks; hormone levels and CT scans were obtained every 6 to 10 weeks in selected cases.

Response criteria included the following: complete remission (CR), disappearance of all evidence of disease for 1 month; partial remission (PR), $\geq$50% decrease in the summed products of the perpendicular diameters of all measurable lesions and a $\geq$50% decrease in all abnormally elevated biochemical parameters; minor response (MR), 25% to 49% objective decrease of all measurable parameters; stabilization of disease (STAB), $\leq$25% change (increase or decrease) for 3 months; and progression (PROG), $\geq$25% increase in measurable lesions, or a mixed response. The duration of response and sur-

vival were measured from initiation of therapy. An adequate trial of flutamide required a minimum of 3 continuous weeks of therapy. The Karnofsky performance status was used to evaluate subjective response.

The dose of flutamide was 250 mg by mouth three times a day. The dose was never increased because of reports of gynecomastia and lactation in patients given 1000 mg daily. Patient compliance was monitored by counting the number of pills remaining with the patient at each visit.

Including specimens from 12 patients given flutamide, material from a series of 62 specimens from 44 men and 18 women varying from 33 to 78 years of age was evaluated for the presence of steroid-binding sites. Forty-one specimens were primary renal tumors; 25 were clear cell carcinoma, 7 were renal cell carcinoma with granular or eosinophilic cytoplasm, 5 were high-grade poorly differentiated tumors, and 3 were transitional cell (urothelial) carcinomas. The remaining neoplasm was an oncocytoma. Twenty-one specimens were from proven metastases.

The histochemical technique for the detection of steroid hormone-binding sites has been described previously.[22] In brief, frozen sections of tumor were exposed for 2 hours to 7×10^{-7} mol/l of steroid ligand conjugates. These were synthesized by covalently linking estradiol, hydroxyprogesterone or dihydrotestosterone, with bovine serum albumin (BSA) and then labeling the product with fluorescein isothiocyanate (FTIC). Parallel sections were additionally exposed to a molar excess of specific competitor ligand. For this purpose, nitromifene citrate (CI 628), 7×10^{-6} mol/l was used as the competitor for estradiol, the synthetic progesterone promegestone (R5020), 3.5×10^{-5} mol/l for progesterone, and cyproterone acetate, 3.5×10^{-6} mol/l for androgen. To inhibit the binding of the progesterone ligand to androgen and glucocorticoid receptor, a 100-fold concentration of dihydrotestosterone and hydrocortisone was added. Nonspecific binding of BSA was monitored by incubating other sections in BSA FTIC unlinked to steroid and in the same concentration as the other ligands.

Specimens were designated as positive only when >10% of the component tumor cells exhibited fluorescence, and no binding of BSA FTIC was seen; while in sections exposed to both ligand and competitor there was a reduction of fluorescence by at least 50% when compared with sections exposed to only ligand conjugate.

Patient characteristics are outlined in Table 1. Of note, 48% of cases had a PS of 90 to 100 while only 4% had a PS of $\leq$50, and 75% of the cases had received no prior irradiation or cytotoxic chemotherapy, and 64% had no prior hormonal therapy. Of 13 patients previously treated with either a hormone or cytotoxic drug, none had ob-

tained an objective response. Initially, three patients had an abnormally elevated serum calcium level ($\geq$10.8 mg/dl) and 6 an elevated BUN ($\geq$20 mg/dl) or a serum creatinine (1.4 mg/dl) level. Ten patients had evidence of pretreatment elevations of liver function tests, *i.e.,* LDH $\geq$ 250 and/or SGOT $\geq$ 25 IU/dl). The indicator lesions were pulmonary nodules in 21 patients, abdominal masses in 10, lymph nodes in 8, hepatic metastases in 6, and subcutaneous masses in 7. All cases had bidimensionally measurable disease; 6 had one parameter, 10 had two, and 9 had three or more tumor sites. In addition, two patients had osseous metastases. Patients were started on flutamide in a median of 2 days (range, 0–142 days) from the time of the last biopsy; 19 patients had their biopsy within 2 weeks of starting therapy.

Results

Twenty-five (89%) of the 28 patients had an adequate trial; 2 refused follow-up evaluation, and 1 died of unrelated causes 19 days after starting therapy.

One (4%, 95% confidence limits 0–12%) of 25 adequately treated patients had a PR lasting 9+ months, and 2 additional patients had stabilization of disease lasting 6 and 15 months, respectively. Twenty-two (88%) patients had progressive disease. The patient with the strongest fluorescence for androgen-binding protein in his carcinoma died of progressive unrelenting disease 3 weeks after starting therapy.

Patients were treated for a median of 45 days (range, 20–453) before progressive disease became evident. Of the patients who received an adequate trial, six patients were on therapy for $\leq$4 weeks, six for 4 to 8 weeks, four for 8 to 12 weeks, and nine for 12 to 66 weeks. The drug was generally well tolerated. In three cases a marked elevation in liver function tests occurred and flutamide was stopped. One patient permitted a percutaneous liver biopsy and no pathologic abnormality was found. One of three patients with pre-existing hypercalcemia had an unexplained increase in the calcium level. Galactorrhea or gynecomastia were not observed.

In selected patients, luteinizing hormone (LH) and testosterone determinations revealed no definitive effect of flutamide. The level of LH increased over the baseline level in 33% of cases and decreased in 67%. The patient's gender did not correlate with the probability of increasing or decreasing LH levels. Serum testosterone was similarly affected, increasing in 45% and decreasing in 55% of cases. Again, no predictable changes on the basis of the patient's sex were noted.

In 23 patients biopsied, an adequate sample for steroid-binding-site determination was obtained in 12 cases. An estrogen-binding site was demonstrated in six, an andro-

TABLE 1.   Characteristics of 25 Adequately Treated Patients Given Flutamide

Males (%)	64
Age in years (median)	59
	(24–78)
PS (median)	80
	(50–100)
Prior therapy (%)	
Nephrectomy	68
Chemotherapy	40
Irradiation	28
Hormone	28
Immunotherapy	8
Histology (%)	
Adenocarcinoma	48
Clear cell carcinoma	44
Adenocarcinoma & spindle	
cell sarcoma elements	8
History in mo (median)	
Symptoms to diagnosis	1
	(0–16)
Diagnosis to metastases	0
	(0–74)
Diagnosis to protocol	4
	(0–81)

( ): range.

gen binding site in three (borderline positive in two of these three cases), and a progesterone binding site in four. An adequate sample was not obtained at the time of percutaneous transthoracic needle biopsy in the one patient who responded.

Results of the histochemical steroid-binding assays in renal cell carcinoma site of biopsy specimen and histologic diagnosis are summarized in Tables 2 and 3.

Discussion

Flutamide demonstrated no significant antitumor activity in patients with disseminated renal cell carcinoma. Since the current study did not obtain a sufficient number of cases of hypernephroma with androgen-binding positivity, we cannot exclude the possible utility of flutamide in such cases.

TABLE 2.   Results of Histochemical Steroid-Binding Assays

Steroids bound*	Primary tumors	Metastases
E+, Pg+, DHT+	6	3
E+, Pg+, DHT–	6	3
E+, Pg–, DHT–	2	2
E+, Pg–, DHT+	2	1
E–, Pg+, DHT+	1	0
E–, Pg+, DHT–	3	0
E–, Pg–, DHT–	21	12
Total	41	21

For the ligand conjugate estradiol (E), progesterone (P), and dihydrotestosterone (DHT) in primary and metastatic renal cell carcinomas.
* >10% of the tumor cells positive.

TABLE 3. Steroid binding of ligand conjugate of Estradiol, Progesterone, and Dihydrotestosterone

Steroids bound	Clear cell carcinoma	High-grade carcinoma	Oncocytoma	Renal-cell carcinoma	Transitional-cell carcinoma
E+, Pg+, DHT+	2	0	1	2	1
E+, Pg+, DHT−	4	2	0	0	0
E+, Pg+, DHT−	1	0	0	1	0
E+, Pg−, DHT+	0	1	0	1	0
E−, Pg+, DHT+	1	0	0	0	0
E−, Pg+, DHT−	2	0	0	1	0
E−, Pg−, DHT−	15	2	0	2	2
Total	25	5	1	7	3

In primary renal cell carcinomas by histochemistry: relation to histologic diagnosis.

E: estradiol; Pg: progesterone; DHT: dihydrotestosterone.

In prior biochemical steroid receptor assays of renal cell carcinomas, estrogen receptors, and/or progesterone receptors have been reported in 61%[14] and androgen receptors in 75%.[23] In the current series, estrogen binding was only detected in significant proportions qualitatively in 40%, progesterone in 34%, and androgens in 21% of the 62 specimens. Discrepancies between these and other results may be largely a matter of differences in histochemical and biochemical technique. One of the virtues of histochemistry is that heterogeneity of tumor cells in carcinomas is obvious. Since the large majority of renal cell carcinomas are highly cellular, it is possible that a cytosol derived from myriads of cells relatively low in receptor might contain sufficient amounts of specific binding proteins to be biochemically detectable. The failure of nearly all of the patients to respond to flutamide may indeed be related to the low proportion of tumors exhibiting significant positivity as judged histochemically. It is still unclear whether histochemical techniques identify the same hormonal binding proteins as are customarily measured biochemically.[24,25] It has been clearly shown that histochemical binding is target-organ-specific, correlates extremely well with biochemical receptor assays in both breast and prostate cancer, and with clinical response to hormonal treatment.[26,27] Furthermore, binding is competed for by specific competitor ligands, even though, in concentrations higher than used by the biochemists, and the observed fluorescence can be translocated from cytoplasm to nucleus *in vitro* as would be expected of classical receptor sites.[25]

Recently, Nakano and associates[28,29] reviewed their experience with estrogen, progesterone, and androgen receptors in normal kidney and hypernephroma specimens. Hormone receptors were found both in normal renal tissue and in renal cell carcinoma, but the level of hormone receptor declined progressively with an increasing grade of malignancy. Although both normal and malignant renal tissue has detectable quantities of hormone receptors

the presence of hormone receptors does not seem to correlate with hormonal responsiveness.

No well-controlled clinical trials have shown, except for isolated cases, significant efficacy with hormonal therapy. Newer methods of treatment still need to be explored.

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