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PROGESTATIONAL THERAPY IN HUMAN RENAL CARCINOMA AND STEROID RECEPTORS

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SUMMARY

Normal human kidney and human renal adenocarcinoma have been studied for progesterone and estradiol receptors by means of agar gel electrophoresis technique. Progesterone receptor was found in the cytosol fraction of all the normal kidneys and of the adenocarcinoma studied, while estradiol cytosol receptor was found only in some of the tissues examined.

No correlation between the degree of the differentiation of the tumours and the progesterone binding capacity has been detected. Furthermore the binding affinity for estradiol was greater than that for progesterone.

The low affinity of progesterone receptor could explain the large mass (pharmacological doses) of progesterone needed to obtain a marked tumour regression as well as a favorable effect on the clinical course of the advanced renal cancer.

Like human breast cancer, responsive to endocrine ablation when both estradiol and progesterone receptors are present, it could be hypothesized that progestational therapy may be useful in the treatment of renal adenocarcinoma in which both estradiol and progesterone receptors are present.

The presence of steroid receptors in the kidney has recently been reported by several authors [1–5]. In previous investigations using Dextran-coated charcoal and agar gel electrophoresis the presence of estradiol and progesterone receptors was demonstrated in normal human kidney and in human renal adenocarcinoma [6, 7]. These studies were performed to investigate the mechanism of action involved in the experimental and clinical results of the progestational therapy used in advanced human renal carcinoma. These neoplasias have been favorably treated with progestational compounds, not only in the more advanced forms and those with metastases, but also as a preventive measure to avoid secondary diffusion following surgery [8–13].

EXPERIMENTAL

Biological material

Kidney specimens, obtained during surgery by Dr. F. Di Silverio (Urology Department of the University of Rome), were immediately frozen on dry ice and stored at −22°C until processed.

Radioactive material

The following radioactive compounds were used:
[2,4,6,7-^{3}H]-estradiol 100 Ci/mmol.
[1,2,6,7-^{3}H]-progesterone 100 Ci/mmol.
[15,16-^{3}H]-D-norgestrel (13β-ethyl-17α-ethinyl-17β-hydroxy-19Nor-androst-4-en-3-one) 56 Ci/mmol.

Radiochemical purity was determined by paper or t.l.c.

Methods

Kidney specimens were cut into small pieces, rinsed with 0.9% NaCl, minced with scissors and homogenized for 10 s in two vol. of 0.01 M Tris–HCl buffer pH 7.5, 0.001 M NaN_3 or 0.01 M Tris–HCl buffer pH 7.5, 0.0015 M EDTA, 0.5 mM dithiothreitol (DTT). DTT was added freshly each time it was used. The homogenate was centrifuged at 10,000 *g* for 30 min at 2°C and the supernatant centrifuged again at 200,000 *g* for 90 min at 2°C. The resulting supernatant (cytosol) was divided into 1 ml portions and the protein concentration determined by the Folin phenol method of Lowry *et al.* [14].

Cytosols, tested for progesterone receptor with [^{3}H]-progesterone, were preincubated with cortisol ($1–10 \times 10^{-6}$ M) to saturate the binding sites of glucocorticoid receptors and corticosteroid binding globulin (CBG), and with a 100-fold excess of cortisol when tested with [^{3}H]-D-norgestrel which shows little affinity for CBG.

Details of the method used have been reported elsewhere [7].

RESULTS

Estradiol receptor

The presence of an estradiol cytosol receptor was demonstrated in only one of the three normal kidneys examined. Figure 1 shows the migration of the [^{3}H]-estradiol macromolecular complex in the anodic area and the effect of the addition of 100-fold excess of cold estradiol-17β.

In five of the ten renal adenocarcinoma examined it was possible to demonstrate the presence of a specific estradiol cytosol receptor (Fig. 2).

Progesterone receptor

A specific progesterone cytosol receptor was demonstrated in the three normal human kidneys.

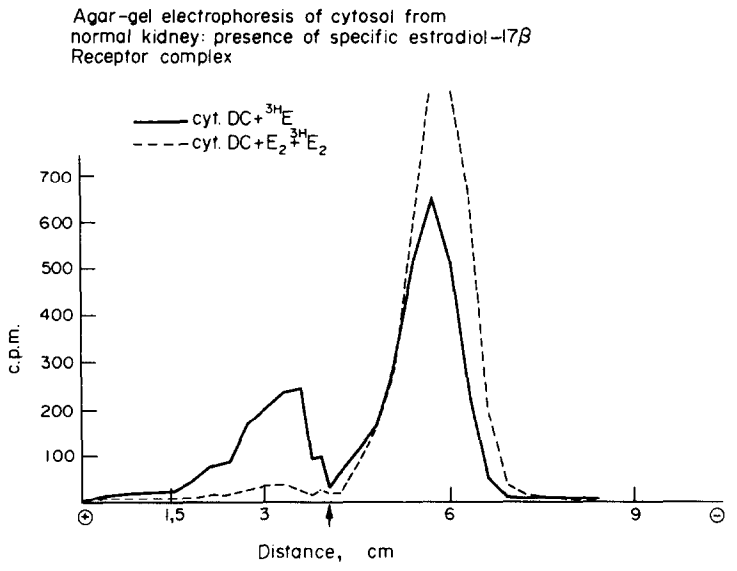

Fig. 1. Agar gel electrophoresis of cytosol from normal human kidney: presence of specific estradiol receptor complex. Incubation with 1×10^{-9} M [^{3}H]-estradiol-17β for 18 h at 4°C (solid line); incubation with 1×10^{-9} M [^{3}H]-estradiol-17β plus 1×10^{-7} M cold estradiol-17β for 18 h at 4°C (dotted line). 1% agar gel in 0.05 μ sodium diethyl-barbiturate/acetate buffer pH 8.2; run 280 V for 3 h; 50 μl samples applied for analysis, 60 sections of 1.5 mm.

A specific progesterone receptor was found in all but one of the ten tumours examined (Fig. 3). Figure 4 shows the migration of the [^{3}H]-D-norgestrel macromolecular complex in the anodic area and the effect of the addition of 100-fold excess of cold D-norgestrel.

Concentration of estradiol and progesterone receptors

Pretreatment of the cytosol with a suspension of Dextran-coated charcoal (0.5% Norit A, 0.05% Dextran) led to an increase in the percent of tritiated estradiol recovered in the anodic area (from 21.04 to 44.79%). No substantial difference was found between freshly worked specimens and specimens frozen for 3 days (44.79 vs 42.41%). The addition of DTT had a protective effect on the binding of estradiol, leading to an increase of the number of binding sites, as demonstrated by quantitative experiments.

A Scatchard plot of the specific binding of estradiol and progesterone in normal human kidney and in human renal adenocarcinoma was performed to measure the number of binding sites and the dissociation constant. Figure 5 illustrates the Scatchard analysis for progesterone receptor in one renal adenocarcinoma.

Although only a few normal renal specimens have been examined, the results of quantitative determinations on kidney carcinoma clearly demonstrate a larger number of binding sites for progesterone than that found in normal kidney (see Table 1).

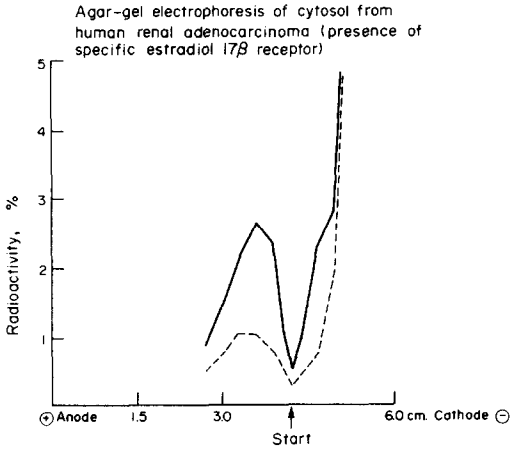

Fig. 2. Agar gel electrophoresis of cytosol from human renal adenocarcinoma (T_7): presence of specific estradiol receptor complex. Incubation with 1×10^{-9} M [^{3}H]-estradiol-17β for 18 h at 4°C (solid line); incubation with 1×10^{-9} M [^{3}H]-estradiol-17β plus 1×10^{-7} M cold estradiol-17β for 18 h at 4°C (dotted line). 1% agar gel in 0.05 μ sodium diethyl-barbiturate/acetate buffer pH 8.2; run 280 V for 3 h; 50 μl samples applied for analysis, 30 sections of 3.0 mm.

PATIENTS	E_2 - R	P - R	SEX	P *	METASTASES
T_1	-	+	♀	P2	Mo
T_2	+	+	♂	P3a	Mo
T_3	-	+	♀	P2	Mo
T_4	+	+	♂	P3a	Mo
T_5	+	+	♂	P2	Mo
T_6	-	+	♂	P2	Mo
T_7	+	+	♂	P3b	Mo
T_8	-	+	♂	P3b	Mo
T_9	-	-	♂	P3a	M1c
T_{10}	+	+	♀	P2	Mo

* T.N.M. (U.I.C.C - Geneva 1974)

Fig. 3. Presence of estradiol and progesterone receptors in cytosol from human renal adenocarcinoma with different stages of invasion.

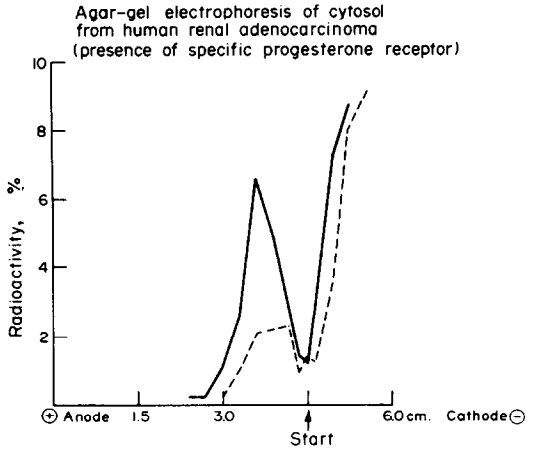

Fig. 4. Agar gel electrophoresis of cytosol from human renal adenocarcinoma (T_7): presence of a specific progesterone receptor complex. Cytosol preincubated with 1×10^{-7} M unlabelled cortisol. Incubation with 1×10^{-9} M [^{3}H]-D-norgestrel for 18 h at 4°C (solid line); incubation with 1×10^{-9} M [^{3}H]-D-norgestrel plus 1×10^{-7} M cold D-norgestrel for 18 h at 4°C (dotted line). 1% agar gel in 0.05 μ sodium diethylbarbiturate/acetate buffer pH 8.2; run 280 V for 3 h; 50 μl samples applied for analysis, 30 sections of 3.0 mm.

Quantitative analyses on a larger number of specimens are needed, but on the basis of results so far obtained it is evident that the behaviour of the tumours was different from that of normal human kidney, as far as the number of binding sites and the dissociation constant are concerned. In fact, first of all, in the cytosol of normal human kidney the binding capacity for estradiol is higher than that for progesterone and the dissociation constant is of the same order for both receptors; second, in the cytosol of human renal adenocarcinoma the binding capacity for estradiol is lower than that for progesterone, but the estradiol receptor shows a higher affinity than progesterone receptor; third, while no substantial difference was found between human renal cancer and normal human kidney, as far as concerned the capacity and the affinity of estradiol receptor, in the cytosol of human renal adenocarcinoma the binding capacity for progesterone receptor is twice or three times higher than in the cytosol of normal human kidney, and the affinity of progesterone for its receptor is ten times lower than that measured in normal kidney.

DISCUSSION

The use of hormonal therapy in the management of renal adenocarcinoma was introduced as soon as

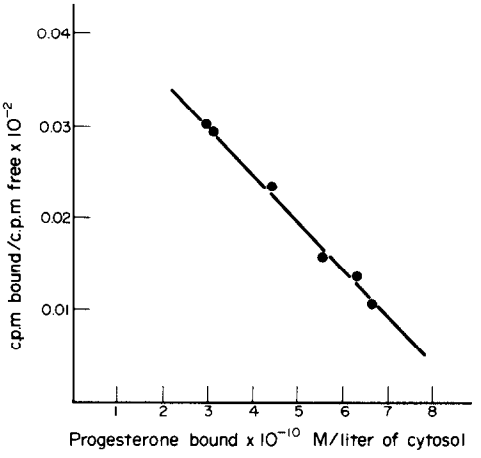

Fig. 5. Scatchard plot of progesterone cytosol receptor from human kidney tumour (T_2). Cytosol preincubated with 1×10^{-6} M unlabelled cortisol. Specific binding determined by the addition of 1000-fold excess of cold progesterone.

Table 1. Binding sites and dissociation constant of estradiol and progesterone receptors in normal human kidney and in human renal adenocarcinoma derived from Scatchard plot

	E_2		Prog.	
	n (F MOLES/MG. PROTEIN)	Kd (10^{-9} M)	n (F MOLES/MG.PROTEIN)	Kd (10^{-9}M)
Normal case 2	32.04	7.40	11.22	3.60
Normal case 3	-	-	39.83	64.30
T1 ♀	-	-	144.00	39.50
T2 -DTT	13.42	0.13	-	-
T2 +DTT	71.67 166.67	2.61 31.00	73.33	19.20
T3 ♀	-	-	25.00 155.74	0.47 45.10
T4	32.73	3.61	7.73	2.31
T5	5.70	0.89	138.16	21.00
T6	-	-	196.00	51.60

it was realised that this neoplasia was hormone-dependent.

Hormones have not been found to induce renal adenocarcinoma in species other than hamsters [15], nor have they been shown to function as an etiologic agent in naturally occurring renal adenocarcinoma. Nevertheless, an attempt was recently made to explain on a molecular basis the possibility that estrogens play a role in the genesis of human renal adenocarcinoma. In fact, as already demonstrated in Syrian hamsters [16–19] estrogens may act simply as cocancerogenic compounds, unmasking the DNA copy of the C type RNA virus [17], which would then promote the formation of viruses. It is important in this regard to recall that human RNA virus particles have been detected by electron microscopy in papillary tumours of the human renal pelvis [20] but not yet in renal adenocarcinoma although a latent oncogenic virus could be present as in the monkey kidney.

Some clinical observations such as the significant sex incidence of human renal adenocarcinoma (twice as common in men as in women) and its variation with the cessation of gonadal activity, the racial differences, the regression of metastatic renal cancer during the adminstration of progestin or androgen (greater in men than in women), occasional tumour progression following hormone treatment, led to the hypothesis of a hormone-dependence in human renal adenocarcinoma [9]. Between 1959 and 1971 Bloom used medroxyprogesterone acetate to treat patients with inoperable renal carcinoma, and obtained a 16% rate of tumour remission [8]. Bracci and coworkers, using progestational compounds as a postoperative adjuvant in the more advanced form of renal carcinoma or in those with metastases, reported a significant difference between treated and non treated patients [10–12].

A large amount of data reported in the literature confirms that hormonal treatment may be the treatment of choice in the management of metastatic renal hypernephroma [21]. According to other authors [22, 23] no objective response was obtained after either progestagens or androgens, and the remission rate of 20% in cases of metastatic hypernephroma with progestational agents was considered a spontaneous regression incidence of this tumour. Alberto and Senn [24], on the other hand hypothesize that the differences between their own results and those of others could possibly be explained on the basis of the administration of lower doses of progestagens compared with those of Bloom.

The present studies were performed in an attempt to ascertain if the different response to progestational therapy could be related to the presence of steroid receptors.

Organs such as kidney may be considered "non target" tissues which possess steroid receptors with the same characteristics as those found in the "target" cells [1–5]. Competition between different hormones at receptor level may well be significant since progesterone, deoxycorticosterone and testosterone propionate inhibit the formation of estrogen induced renal tumours [25] in Syrian hamster kidney, which possess both estradiol and progesterone receptors [26, 27].

In agreement with the results of other authors [5, 28], the present data are in favour of the presence of estradiol and progesterone receptors in normal human kidney as well as in human renal adenocarcinoma. Nine of the ten tumours examined had a progesterone receptor and five had an estradiol receptor. Only six of the ten patients have been on progestational treatment for one year: therefore to date the relationship between possible objective remissions and presence of steroid receptors cannot be evaluated. The only tumour with neither progesterone nor estradiol receptors was in a patient with lung metastases; nevertheless progestational therapy was started also in this patient. No correlation was found between the degree of differentiation of the tumours or its state of invasion and the progesterone or estradiol receptors.

The relationship between sex ovarian hormones and estradiol and progesterone receptors is well known: both progesterone in the luteal phase and progesterone treatment induce a decrease of the binding capacity of endometrial estradiol receptor. Both estrogen treatment and exposure to estrogens induce an increase in progesterone receptor concentration [29–34].

Like human breast cancer which is unresponsive to endocrine ablation in the absence of estradiol receptor, it could be hypothesized that progestational therapy may be useful in the treatment of renal adenocarcinoma in which both estradiol and progesterone receptors are present.

In estrogen target tissue the synthesis of progesterone receptor depends on the action of estrogens [35]. It could be hypothesized that progesterone action is antagonistic to that of estradiol not only on target cells but also at the level of other tissues where these receptors are present.

An imbalance between progesterone and estradiol, characterized by progesterone insufficiency may play a role in the pathogenesis of cancer [36]. Progesterone influences the binding of estradiol possibly through a competitive inhibition mechanism [31]. However we were unable to demonstrate that progesterone is even a weak competitor for estradiol receptor.

In a previous investigation the increased CBG level in five out of six tumours examined was considered as an indirect index of estrogen action on these patients.

In the treatment of these tumours large mass (pharmacological doses) of progestins may be required to induce a marked tumour regression. Favorable results may be expected mainly in those tumours which have both progesterone and estradiol receptors.

The discrepancies between the results obtained with progestational therapy in human renal adenocarcinoma may be merely due to the choice of dosage. The large amount of progesterone needed to create a competitive mechanism between progesterone and estradiol receptors on the specific regulator effect of nuclear RNA metabolism may be explained on the basis of the high affinity of CBG for progesterone and the low affinity of progesterone receptor. Furthermore large doses may be required if progesterone is only a weak competitor for the estradiol receptor. Finally, progestational compounds have been used in preference to androgens since an aromatization process appears to affect these steroids.

Progesterone action may be mediated by androgen receptors in the kidney [37] and the progestin effect may be mediated by androgen receptors following *in vivo* conversion to potentially active androgens [38]. Recent unpublished results obtained by Bullock *in vivo* and Bardin *et al.* [39] in *in vitro* experiments, indicate that [^{3}H]-MPA binds directly to the kidney androgen receptors. Recently Bracci and coworkers have been using gestonorone capronate (19α-nor-17 hydroxy-progesterone capronate), a steroid without virilizing, anabolic, feminizing, antiandrogen activity.

Competition experiments are being carried out between different steroids at receptor level which might be involved in the kidney, meanwhile it is worthwhile remembering that the discrepancies between the results obtained with progestational therapy in human renal carcinoma may be related merely to the choice of dosage.

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