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Studies on Progesterone Receptors in Human Breast Carcinomas: Use of Natural and Synthetic Ligands*

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Abstract—*Progesterone receptors were measured in human breast carcinomas using both* (^{3}H)*progesterone in the presence of excess non-radioactive cortisol and tritiated synthetic progestins which are not bound with high affinity by corticosteroid-binding-globulin. At concentrations of receptor above* 10 fmole/mg *protein good agreement was found between the amount of progesterone and the synthetic progestins R5020 and ORG* 2058 *bound. In* 2/27 *tumors low levels of progesterone binders were present but no specific binders for the synthetic progestins were detected. In all the tumors without specific progesterone binders, receptors for both R5020 and ORG* 2058 *were also absent. The* K_d *using the different ligands were in the order R5020 < ORG* 2058 *< progesterone in the presence of glycerol < progesterone in the absence of glycerol. Competition experiments using excess non-radioactive steroids suggest that both progesterone and the synthetic progestins bind to identical sites. It is concluded that neither* (^{3}H)*progesterone nor* (^{3}H)*ORG* 2058 *offers any advantage over R5020 in the assay of progesterone receptors in human breast tumors.*

INTRODUCTION

It is now well established that approximately 50–60% of human breast carcinomas containing estradiol receptors (ER) respond to endocrine manipulative therapy while less than 10% ER-negative tumors regress [1, 2]. If however, progesterone receptors (PgR) are present in addition to the ER, a greater proportion of patients benefit from the hormonal treatment [3, 4]. It is therefore desirable to assay PgR simultaneously with ER in human breast tumors.

Until recently accurate quantitation of PgR had been difficult. In addition to progesterone binding to its own receptor, it also binds with relatively high affinity to corticosteroid-binding-globulin (CBG) [5] which invariably contaminates human breast tumors [6]. Furthermore using (^{3}H)progesterone as ligand the receptor–steroid complex dissociates rapidly during the separation procedures [7] thus underestimating receptor concentration.

These problems have been largely overcome by using either (a) synthetic (^{3}H)progestins which are not bound with high affinity by CBG and which dissociates relatively slowly from the receptor [3, 4, 8, 9] or (b) by using (^{3}H)progesterone as ligand and blocking CBG with excess radioinert cortisol [10, 11]. Using the latter procedure the half-life of the progesterone–receptor complex is considerably increased in the presence of glycerol [11].

In this paper we compare the results obtained on PgR measurements in human breast tumors using 2 different synthetic (^{3}H)progestins as ligands with those obtained using (^{3}H)progesterone. Furthermore we present evidence suggesting that both the synthetic progestins and progesterone are bound by the same binding protein.

MATERIALS AND METHODS

Materials

(^{3}H)progesterone was purchased from the Radiochemical Center, Amersham, England.

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(^{3}H)R5020 (17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione) and non-tritiated R5020 were gifts from Dr. J. P. Raynaud, Roussel-Uclaf. (^{3}H)ORG 2058(16α-ethyl-21-hydroxy-19-nor-4-pregnene-3,20-dione) and non-radioactive ORG 2058 were generously supplied by Dr. E. de Jager, Organon. All other non-radioactive steroids were purchased from Sigma Chemicals Ltd.

Methods

Tumors were obtained from patients operated on in St. Vincent's Hospital and were stored in liquid nitrogen for periods not greater than 2 weeks, Homogenisation of tissue and preparation of cytosols was as previously described [12, 13].

Progesterone receptor assay

(a) *Using* (^{3}H)*R5020*. Cytosols were incubated for 18 hr at 4°C with increasing concentrations (5×10^{-8}M – 5×10^{-9}M) of (^{3}H)R5020 in the presence and absence of 500-fold excess non-tritiated R5020.

(b) *Using ORG* 2058. Method was identical to above except ORG 2058 was used instead of R5020.

(c) *Using* (^{3}H)*progesterone*. This procedure was based on the method of Pichon and Milgrom [11]. Binding to CBG was blocked using excess non-radioactive cortisol. Incubation was also carried out for 18 hr at 4°C. One hour before termination of reaction, glycerol was added to the incubation medium giving a final concentration of 30%. The dextran–charcoal suspension also contained glycerol at a final concentration of 30%.

For each ligand, binding was terminated by the addition of 0.5% charcoal-0.05% dextran [13]. The tubes were vortexed, allowed to stand at 0°C for 10 min and centrifuged at 2000 ***g*** for 10 min. The supernatant was decanted and counted using 0.5% PPO in toluene: triton-X-100 (2:1; V; V) as scintillant. The binding data was plotted as described by Scatchard [14] and the K_d was calculated from the slope of graph.

Protein measurement. Protein was determined as previously described using bovine serum albumin as standard [15].

RESULTS

Table 1 shows the concentration of binding proteins in 27 primary breast carcinomas using the 3 different progestins. Except for patients numbered 5 and 11 there is good agreement between the concentrations using the different ligands. Tumors from these 2 patients contained low levels of progesterone binding proteins but no R5020 or ORG 2058 binding sites. The progesterone binding components in these tumors were not precipitated by protamine sulfate [16] suggesting binding was not due to specific receptors. On the other hand, 3 tumors with low concentrations of binding sites for both progesterone and the synthetic progestins using the charcoal method of separation did contain specific progesterone binding proteins precipitated by protamine sulfate e.g., tumors numbered 6, 9 and 15 contained 12, 6, 9.5 and 18 fmole/mg protein respectively. All of the tumors without specific progesterone receptors also lacked both R5020 and ORG 2058 receptors. The average K_d for the 3 ligands were in order R5020 < ORG 2058 < progesterone in the presence of glycerol < progesterone in the absence of glycerol (Table 2).

Table 1. *Concentration of progestin binders in human breast tumors using* 3 *different tritiated progestin ligands*

Patient No.	Concentration of binder (fmole/mg protein)		
	Progesterone	R5020	ORG 2058
1	28	32	35
2	106	123	106
3	33	29	26
4	536	428	471
5	8.5	0	0
6	15	11	9
7	22	28	31
8	138	146	127
9	8.4	12	15
10	98	109	84
11	6.8	0	0
12	57	44	68
13	27	21	23
14	39	47	44
15	11	19	12
16 27	0	0	0

Procedure as described in section on Methodology.

Table 2. *Average* K_d *for progestin binding proteins in human breast tumors using different progestin ligands*

Ligand	K_d(nM)
R5020	0.95 (13)
ORG 2058	1.08 (13)
Progesterone plus glycerol	1.20 (13)
Progesterone minus glycerol	4.25 (5)

Number of demonstrations given in parentheses.

Competition experiments with excess non-tritiated steroids suggest that both progesterone and the synthetic progestins bind to the same binding site (Table 3). Excess (100-fold) of each of the non-radioactive progestins inhibited binding of the 3 labelled progestins. On the other hand excess radioinert estradiol, dihydrotestosterone and cortisol had no significant effect on the binding of the tritiated ligands.

Table 3. Effect of excess non-radioactive (100-fold) steroids on the binding of (^{3}H) progestins by human breast tumors

Unlabeled steroid	Inhibition of binding of (^{3}H)progestin (%)		
	(^{3}H)progesterone	(^{3}H)R5020	(^{3}H)ORG 2058
Estradiol	1.7	0	0
Dihydrotestosterone	2.0	1.4	1.8
Cortisol	3.6	0	0
Progesterone	100	85	82
R5020	96	100	98
ORG 2058	93	97	100

Procedure as carried out in section on Methodology. Results are means of 3 determinations.

DISCUSSION

The 3 different progestins used in the present investigation have each been used separately to measure PgR in human breast tumors [3, 4, 8, 9, 10, 11]. Our results show that when PgR concentrations are greater than about 10 fmole/mg protein, good agreement is found with the different ligands. However at low levels of receptor, binding by serum proteins when using (^{3}H)progesterone may give false receptor positive values. In this context also, Raynaud *et al.* [17] found the greatest discrepancy between the amount of progesterone and R5020 bound at low concentrations of receptor. At low levels of receptor use of tritiated synthetic ligands may therefore lead to more specific binding.

Potential disadvantages in the use of both R5020 and ORG 2058 include non-specific binding of both ligands to unidentified serum proteins [4, 9] and the binding of R5020 at least to the glucocorticoid receptor (GR) [18]. However the low affinity non-specific binding to serum proteins can be differentiated from specific receptor binding by saturating the receptor with excess radioinert progestin and/or determining the K_d of the ligand–protein interaction. On the other hand binding of R5020 to the GR might be inhibited by the addition of excess non-radioactive cortisol. Further investigations are necessary to confirm this.

In conclusion although both progesterone and synthetic progestins can be used to assay PgR in breast tumors the final evaluation of which ligand is best must await correlation with the response of tumors to endocrine therapy.

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