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Presence of Progesterone Receptors and Absence of Oestrogen Receptors in Human Intracranial Meningioma Cytosols*†

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Abstract—*The occurrence of oestrogen and progestin receptors in cytosols from human intracranial meningiomas was studied with a dextran-coated charcoal assay and Scatchard plot analysis.* [^{3}H]-*Oestradiol and* [^{3}H]-*promegestone* (17α,21-*dimethyl*-19-*norpregna*-4,9-*diene*-3,20-*dione, R*-5020) *were used as tracers. Using this method, no high-affinity binding sites for oestradiol were observed, whereas progestin binding was identified in* 18 *out of* 20 *meningioma cytosols. The number of progestin binding sites was identical in meningioma cytosols obtained from female patients* (192 ± 57 fmol/mg *protein, mean* ± *S.E.M.*, $n = 12$) *and those obtained from male patients* (230 ± 57 fmol/mg *protein*, $n = 6$), *as was the dissociation constant of the complex* (1.5 ± 0.3 *vs* 1.4 ± 0.3 nmol/l *respectively*). *Only progestins (progesterone, R*-5020 *and megestrol acetate) competed successfully with tritiated R*-5020. *Oestrogens, androgens and cortisol showed no appreciable cross-reaction. It was concluded that the cytosols from human intracranial menigiomas contain progesterone receptors in the absence of oestrogen receptors. The presence of these progesterone receptors may indicate that (anti)-progestational treatment could be of potential value in cases which cannot be treated by surgery alone.*

INTRODUCTION

THE INCIDENCE of meningiomas in women is higher than in men and meningiomas have been reported to proliferate faster in patients with increased circulating levels of sex steroids, i.e. in pregnant patients. These observations have prompted several investigators to study the occurrence of binding sites for female sex steroids in meningioma tissues [1–6], but the results of these studies were not unequivocal. Donnell *et al.* [1], Poisson *et al.* [2], Martuza *et al.* [3] and Yu *et al.* [4] have reported the presence of an oestrogen binding principle in meningioma tissue. The concentrations of this binding principle were extremely low, and its affinity for oestrogens was not determined. Yu *et al.* [4] studied the steroid specificity of the binding observed and found that a 100-fold excess of either progesterone, cortisol, testosterone or dexamethasone was not able to compete with 20 nM of tritiated oestradiol. Because of the extremely low concentration of the binding principle and the fact that proper Scatchard plots could not be constructed [3], it seems premature to term this binder an oestrogen receptor.

The occurrence of a progestin binder in the cytosol from meningioma tissue was reported by some of the authors [2–4], but again no data were given on the affinity of the binding. Yu *et al.* [4] showed that progesterone was a good competitor for the binding of R-5020, that oestradiol and testosterone competed to a lesser extent and that corticosteroids competed even less. The capacity of cytosol from meningiomas to bind tritiated R-5020 was higher than that for oestradiol [2, 4].

Schnegg *et al.* [5] and Tilzer *et al.* [6] were the first to report on the affinity of the binding of R-5020. In relatively small series, of 10 and 6

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tumours respectively, they found a K_d of 0.3–10 nmol/l, which is in the range reported for receptor proteins. They were unable to identify oestrogen receptors in their samples.

From the above considerations it appears that meningioma tissue may be quite unique in having progesterone receptors in the absence of oestrogen receptors. The results reported in the present paper for a series of 20 patients are in agreement with this hypothesis.

MATERIALS AND METHODS

Tissues

After excision of the tumours from the patients, a representative sample was submitted for microscopic examination and classification. The remainder of the tissue was immediately frozen in dry ice and transported to the laboratory. Tissues were stored at -80°C until processing.

Radioactive steroids

Tritiated oestradiol-17β (sp. act. 115 Ci/mmol) and R-5020 (17α,21-dimethyl-19-norpregna-4,9-diene-3,20-dione, promegestone, sp. act. 87 Ci/mmol) were purchased from New England Nuclear (Dreieich, F.R.G.). The purity of these compounds was greater than 98% as determined by thin-layer chromatography.

Receptor assays

Receptor assays were performed according to the guidelines of the EORTC Breast Cancer Cooperative Group [7, 8]. Briefly, tissue was pulverized with a Mikrodismembrator (B. Braun, Melsungen, F.R.G.), the tissue powder was extracted with 0.01 M phosphate buffer, pH 7.5, containing EDTA (1.5 mmol/l), sodium azide (3 mmol/l), α-monothioglycerol (10 mmol/l) and glycerol (10% v/v). The suspension was centrifuged at 100,000 ***g*** to yield the cytosol, which was incubated for 16 hr at 4°C with tritiated oestradiol (0.5–10 nmol/l) or R-5020 (0.5–10 nmol/l). In a parallel series of incubations, 10^{-6} mol/l of non-radioactive ligand was present to correct for aspecific binding. Separation of bound and free ligand was achieved by centrifugation after the addition of two volumes of a dextran-coated charcoal suspension (0.25% charcoal and 0.025% dextran T-300, Pharmacia, Uppsala, Sweden) in buffer. Scatchard plots were constructed to calculate the number of binding sites and the dissociation constant of the binder–steroid complexes. The concentration of protein in the cytosols was determined with the method of Bradford [9] using human serum albumin (Kabi, Stockholm, Sweden) as a standard.

A sample was considered to be receptor-positive if (a) a significant correlation in the Scatchard plot was observed, (b) the calculated K_d was less than 5 nmol/l and (c) the protein content of the cytosol exceeded 1 mg/ml.

Competition studies

Competition studies were performed by incubating receptor-positive cytosols for 18 hr at 4°C with 10 nmol/l tritiated ligand and 5–1000 nmol/l of the competitor to be tested. Results were expressed as a percentage of the binding observed in the absence of the competitor.

Statistical analysis

Statistical analysis of data was performed with non-parametric tests (Wilcoxon's test and Spearman rank correlation).

RESULTS

Meningiomas from 6 male and 14 female patients were tested for the presence of receptors. With regard to oestrogen receptors, none of the cytosols fulfilled the criteria A (significant correlation in Scatchard plot) and B (calculated $K_d < 5$ nmol/l) mentioned in the Materials and Methods section. Therefore we concluded that oestrogen receptors are not detectable in human meningioma. By contrast, high-affinity progestin binding was identified in all 6 samples from male patients (Table 1) and in 12 out of 14 samples from female patients (Table 2). As an example, the binding data obtained with cytosol from patient T (Table 1) are shown in Fig. 1. No differences were observed between cytosols obtained from male and female patients with respect to the progestin binding capacity, the dissociation constant of the ligand–binder complex and the amount of protein extracted from the tissue. There was no correlation between the age of the patients and the number of progestin binding sites detected.

To study the steroid specificity of the observed high-affinity binding of R-5020, competition studies were performed with oestrogens [diethylstilbestrol (DES), oestradiol and moxestrol (R-2858)], androgens (testosterone and dihydrotestosterone), progestins (progesterone and megestrol acetate) and cortisol. Meningiomas from patients K and O (Table 2) were used for these experiments. The results shown in Fig. 2 reveal that only progestins effectively compete for the binding of [^{3}H]-R-5020. Therefore we conclude that the high-affinity binder detected in meningioma cytosol is indeed a progesterone receptor.

DISCUSSION

The results of the present study demonstrate that human meningioma tissue is devoid of oestrogen receptors but contains large numbers of

Table 1. Progesterone receptors in cytosols of intracranial meningiomas from male patients

Case	Age (yr)	Cytosol protein (mg/ml)	Progesterone receptor (fmol/mg protein)	K_d (nmol/l)
P	38	4.0	788	2.2
Q	33	6.5	80	0.5
R	70	6.2	158	1.0
S	51	7.2	58	1.0
T	56	7.5	214	1.4
U	74	3.8	84	2.5
n	6	6	6	6
Mean	53.7	5.9	230	1.43
S.D.	16.5	1.6	279	0.77
S.E.M.	6.7	0.7	57	0.31

Table 2. Progesterone receptors in cytosols of intracranial meningiomas from female patients

Case	Age (yr)	Cytosol protein (mg/ml)	Progesterone receptor (fmol/mg protein)	K_d (nmol/l)
A	26	13.9	18	2.8
B	75	10.8	66	4.1
C	62	5.6	95	1.2
D	43	4.2	215	0.4
E	79	7.7	147	1.1
F	62	6.3	460	0.7
G	70	4.7	n.d.*	—
H	39	7.6	12	0.5
J	35	11.2	297	1.5
K	81	3.7	648	1.2
L	66	8.3	32	1.6
M	57	10.9	n.d.*	—
N	73	3.0	66	0.3
O	48	11.4	252	2.3
n	14	14	12	12
Mean	58.3	7.5	192	1.48
S.D.	17.5	3.4	197	1.12
S.E.M.	4.6	1.4	57	0.32

*n.d.: not detectable.

progesterone receptors. Our results are in accordance with those of Tilzer *et al.* [6], who also apply strict criteria for decisions on the presence of receptors in tumor tissue ($K_d < 3$ nmol/l for oestrogen receptors and <10 nmol/l for progesterone receptors), but are in contrast to those of other investigators [1–4]. It is difficult to interpret the extremely low numbers of oestrogen receptors reported by these authors (see Table 3) which were obtained with a single-point assay. Poisson *et al.* [2] reported to extract on the average 60 mg of protein per gram of tissue. This would imply that 20 out of the 22 tissues in their study contain less than 10 fmol/mg protein and should be considered negative for oestradiol receptors. Similar considerations also hold for the data reported by Donnell *et al.* [1] and Martuza *et al.* [3]. The lack of oestrogen receptors reported in the present study is not due to degradation of receptors during storage, since oestrogen binding was not observed in samples stored for as little as one or two days. Moreover, it is generally accepted that the progesterone receptor is more sensitive to degradation upon storage than the oestrogen receptor. It is not likely that occupation of receptors by endogenous oestrogens prevents detection of oestrogen receptors, since these were also not detected in cytosols from tissues obtained from male and postmenopausal female patients (data not shown). Our receptor assay as such is

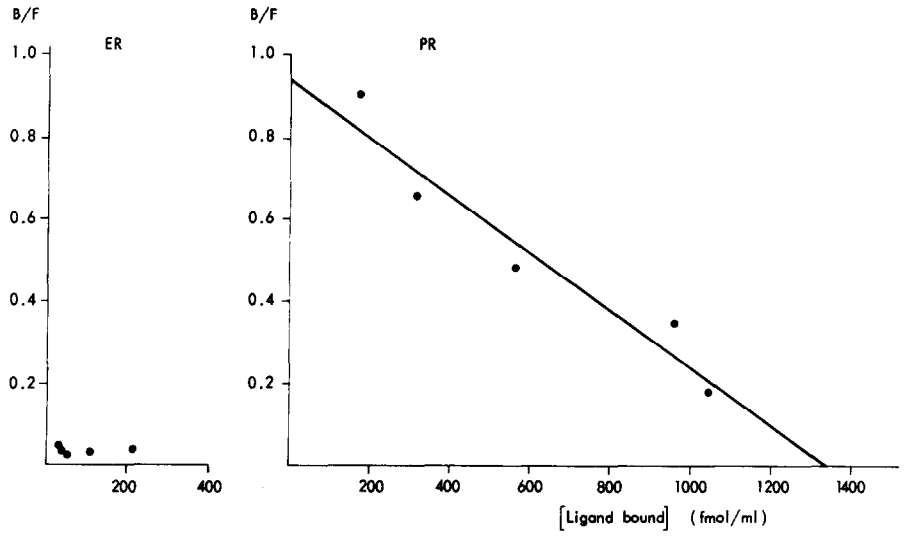

Fig. 1. Scatchard plot analysis of meningioma cytosol from a male patient (T in Table 1) for the presence of oestrogen (ER) and progesterone (PR) receptors.

also not the reason for our inability to detect oestrogen receptors in cytosols from meningioma tissues. In the same period, 63 primary and metastatic mammary tumour tissues from male and pre- and postmenopausal female patients were assayed for the presence of oestrogen receptors. Thirty-three (52%) of these samples were found to contain oestrogen receptors by the criteria used (see Materials and Methods). The validity of the assay was also periodically checked in the Dutch quality control program for steroid

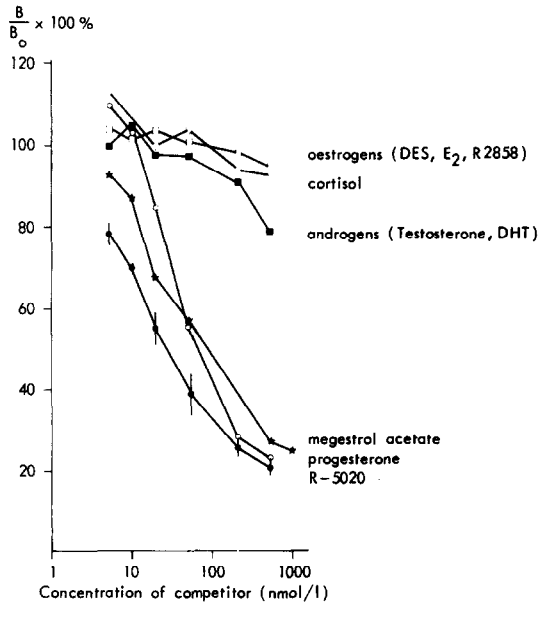

Fig. 2. Competition of progestins, androgens, oestrogens and cortisol for the binding of [^{3}H]-R-5020 in human meningioma cytosol. DES: diethylstilbestrol; E_2: oestradiol; R-2858: 11β-methoxy-17-ethynyl-1,3,5(10)-estratriene-3,17β-diol (moxestrol); DHT: 5α-dihydrotestosterone; megestrol acetate: 17α-acetoxy-6-methyl-pregna-4,6-diene-3,20-dione.

receptor assays [10] and our results were comparable to those of other laboratories.

Our observation that meningioma tissue contains a limited amount of high-affinity binding sites specific for progestins led us to conclude tentatively that these binding sites are progesterone receptors. Theoretically, R-5020 might also bind to androgen or glucocorticoid receptors [11]. Testosterone, 5α-dihydrotestosterone and cortisol, however, are poor competitors for the binding of R-5020 to cytosol from meningioma tissue (Fig. 2). Therefore it is unlikely that the binding observed is due to the presence of other receptors rather than progesterone receptors. Similarly, the steroid specificity of the binding observed (Fig. 2) rules out the possibility that a non-receptor promegestone-binding protein like the one recently discovered in sheep liver cytosol [12] is involved.

It is generally accepted that the synthesis of progesterone receptors is modulated by oestrogens acting through oestrogen receptors. Therefore the occurrence in meningioma tissue of progesterone receptors in the absence of detectable amounts of oestrogen receptors is a remarkable observation. In this respect, meningioma tissue may resemble T47D human breast cancer cells which lack free oestrogen receptors and in which the synthesis of progesterone receptors is not modulated by oestrogens [13, 14]. Conclusions on the functional integrity of the progesterone receptor system in meningioma tissue may be somewhat premature as long as a distinct effect of progestins on this tissue has not been identified. The increased growth rate of meningiomas in pregnant patients, however, is strongly suggestive in this respect. The observed cross-reaction of megestrol acetate

Table 3. Literature data on the occurrence of steroid-binding proteins in human meningioma tissue

Reference	No. of patients	Techniques used	No. of ER^+	Putative ER content	K_d (nM)	No. of PR^+	Putative PR content	K_d (nM)
Donnell *et al.* [1]	6	SGC*	2	212-223 fmol/gT*	—	—	—	—
Poisson *et al.* [2]	22	DCC	13	100-2000 fmol/gT	—	22	100-5000 fmol/gT	—
Martuza *et al.* [3]	10	DCC	7	24-2400 fmol/gT	—	2/3	67-345 fmol/gT	—
Schnegg *et al.* [5]	10	DCC/SPA	0	—	—	4/10	37-355 fmol/mgP	0.3-0.8
Yu *et al.* [4]	16	IEF/SGC	15	1-22 fmol/mgP	—	9/11	13-246 fmol/mgP	—
Tilzer *et al.* [6]	6	DCC/SGC/ DEAE/SPA	0	—	—	4	58-338 fmol/mgP	2-10
Present study	20	DCC/SPA	0	—	—	18	12-788 fmol/mgP	0.3-4.1

*SGC: sucrose gradient centrifugation; DCC: dextran-coated charcoal assay; IEF: isoelectric focusing; DEAE: DEAE-cellulose chromatography; SPA: Scatchard plot analysis; gT: gram tissue (wet weight); mgP: milligram of cytosol protein.

(Fig. 2), a compound which can be of benefit to some breast cancer patients [15], suggests that it may be useful to study the effect(s) of this drug in inoperable meningiomas. Alternatively, the use of the anti-progestational agent RU 38486, which was recently synthesized and characterized [16], should be considered in this respect.

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