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Assay of oestrogen and progestin receptors in human meningioma cytosols using immunological methods

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Summary

Oestrogen (ER) and progestin receptors (PR) were assayed in human meningioma cytosol by radioligand binding assay with Scatchard plot analysis and by monoclonal antibody based enzyme immunoassays. For comparison, human breast cancer tissues were used. Results of both assays agreed very well. For human breast cancer, receptor levels assayed with both methods showed a highly significant correlation (ER: $r = 0.96$; $n = 74$ and PR: $r = 0.95$; $n = 19$). Also for meningioma cytosols a good agreement was observed between the result of both assays. Thus, most meningiomas were devoid of ER but contained significant concentrations of PR. PR levels in meningioma determined with the enzyme immunoassay correlated well with those found by Scatchard plot analysis. After logarithmic transformation of the data the regression line was; PR(EIA) = 0.83 × PR(Scatchard) + 0.36 ($r = 0.917$; $n = 24$). The recognition of the progestin binder in meningioma by a monoclonal antibody against the progestin receptor is a further indication that progestin binding in meningioma occurs to a true receptor.

Introduction

Female sex hormones may be involved in the etiology of human meningioma. This statement is supported by the observations that (1) the incidence of meningioma in women is twice as high as in men, (2) symptoms of meningioma may be aggravated reversibly during periods of relative hormone excess, such as pregnancy and the luteal phase of the menstrual cycle, (3) the occurrence of meningioma is

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associated with that of breast cancer and (4) intracranial metastases of breast cancer show a preference for meningioma over other intracranial neoplasms [1–4].

In the 'classical' target tissues like the uterus and the mammary gland, steroids exert their effects through receptor proteins which, according to the latest views, are located in the nuclear compartment of the cells [5,6]. Moreover, in these tissues, the synthesis of progestin receptors (PR) is stimulated by oestrogens, through the available oestrogen receptors (ER). While most investigators agree that the majority of meningioma tissues are rich in PR some doubt whether the binding observed should be attributed to the presence of a true progestin receptor [7]. In addition, there is still debate on the existence of oestrogen receptors in meningioma. Part of this disagreement may be caused by the use of different methods by the various investigators. Especially the use of 'single point saturation assays' would lead to the classification of meningioma cytosols as ER-positive, whereas investigators using Scatchard plot analysis tend to classify most meningiomas as ER-negative [8,9]. Up to now all investigators have used steroid-binding assays in their efforts to demonstrate the presence of steroid receptors in meningioma tissue. The recent development of monoclonal antibodies against steroid receptors [10,11] and the development of enzyme immunoassays (EIA's) based on these antibodies has enabled a new approach to the subject. We already have reported on efforts to identify oestrogen receptors in human meningioma immunohistochemically [12]. The aim of the present study was to extend these experiments and to investigate whether the progestin receptor from human meningioma would be recognized by antibodies against progestin receptors from other human tissues. The present report describes our experience with the EIA's for ER and PR in the study of human meningiomas.

Materials and methods

Tissues

Meningioma tissue was obtained from the operating theatre and placed on ice immediately. Some tissues were collected in cell culture medium, other tissues were collected dry. After transportation to the laboratory, a representative section of the specimen was frozen at $-80\,^{\circ}C$ and stored at this temperature until processed for Scatchard plot analysis. Remainders of cytosol were frozen at $-80\,^{\circ}C$ for later processing in the enzyme immunoassays. Human breast cancer tissue, which was used for comparative purposes, was obtained from the tissues which were sent to our laboratory for routine assay of ER and PR. These were treated identically to the meningioma tissues.

Ligand binding assays

Assay of oestrogen and progestin receptors were performed according to the European Organization for Research on Treatment of Cancer EORTC Breast Cancer Cooperative Group as described before [13]. Briefly, the tissue was chilled in liquid nitrogen, pulverized with a Mikrodismembrator (B. Braun, Melsungen, FRG) and extracted with 10 mmol/l sodium phosphate buffer, pH = 7.5, containing 1.5 mmol/l EDTA, 3 mmol/l sodium azide, 10 mmol/l 1-monothioglycerol and 10%

(v/v) glycerol. The resulting homogenate was centrifuged at 0–4° C for 30 min at 100 000 × *g* to yield a clear cytosol. [^{3}H]-oestradiol was used as a ligand for the estimation of ER, and [^{3}H]-ORG 2058 for the PR assay. For the estimation of the non-specific binding, excess diethylstilboestrol and ORG 2058 were used respectively. Performance of the assays was monitored by assaying a human myometrium cytosol in every run. Samples were considered to be receptor-positive when a statistically significant correlation was observed in the Scatchard plot, when the dissociation constant did not exceed 5 nmol/l, and when the calculated number of receptor sites was equal to or higher than 10 fmol/mg protein. Results in the range of 3–9 fmol/mg protein were classified as borderline (±).

Enzyme immunoassays (EIA)

Reagents for enzyme immunoassay of ER and PR, were provided by Abbott laboratories (Chicago, IL, USA) and were used according to the instructions of the manufacturer. Briefly, receptors were extracted from the cytosols with polystyrene beads coated with antibodies against the respective receptors; the beads were washed and incubated with a second, enzyme-labelled antibody against the receptor. After the second incubation, the beads were washed again, colour was developed and the extinction of the samples was read at 492 nm. All assays were done in duplicate. Day to day performance of the kits was monitored by assaying control samples provided with the kits in every run. Samples were considered to be receptor-positive by enzyme immunoassay when the assay result exceeded 20 fmol/mg protein.

Protein assay

The protein content of cytosols was estimated with the method of Bradford [14], using human serum albumin (Kabi, Stockholm, Sweden) as a standard.

Results

Performance characteristics of the different receptor assays are given in Table I. From this table, the interassay coefficient of variation of the enzyme immunoassays appears to be somewhat larger than that for the Scatchard plot assays. It should be taken into account, however, that the receptor levels in the control samples used differ considerably, especially for the PR assays. It was concluded that all assays performed properly.

At present, the number of meningioma samples in our series in which ER and PR were assayed by Scatchard plot analysis exceeds 140. Of these 84% contain considerable amounts of PR, whereas in only 9% oestrogen binding fulfilling the criteria stated in the 'Materials and Methods' section was observed. The ER level in those meningiomas selected invariably was very low.

Twenty-one selected meningioma cytosols were assayed for the presence of oestrogen receptors by ER-EIA. Three of these cytosols were ER-positive by Scatchard analysis, 4 were classified as ER ± and 14 were ER-negative. Although 3 samples were thus ER-positive, the ER content of these samples was very low. The individual results for the 21 samples investigated are shown in Table II, together

TABLE I

Interassay variation of radioligand (Scatchard) and enzyme immunoassays (EIA) for oestrogen and progestin receptors [a]

	Scatchard	EIA
Oestrogen receptors		
No. of observations	11	6
Mean (fmol/ml)	216	121
SD	12	15
% CV	5.7	12.6
Progestin receptors		
No. of observations	12	8
Mean (fmol/ml)	1100	60
SD	65	6
% CV	5.9	10.6

[a] For Scatchard analyses a human myometrium cytosol was assayed in every run; for EIA's control samples provided with the reagent kits were used.

TABLE II

Content of oestrogen and progestin receptors in cytosols from human meningioma tissues [a]

Case	ER (Scatchard)	ER (EIA)	PR (Scatchard)
1	0	1	0
2	0	1	60 (+) [b]
3	0	1	283 (+)
4	0	4	0
5	0	1	0
6	0	1	232 (+)
7	22 (+)	5	58 (+)
8	0	6	100 (+)
9	0	1	60 (+)
10	0	1	124 (+)
11	4 (±)	1	27 (+)
12	0	1	6 (±)
13	0	2	35 (+)
14	5 (±)	7	168 (+)
15	0	2	14 (+)
16	5 (±)	13 (±)	26 (+)
17	4 (±)	6	49 (+)
18	0	8 (±)	14 (+)
19	0	4	8 (±)
20	26 (+)	3	97 (+)
21	13 (+)	4	60 (+)

[a] Results are in fmol/mg cytosol protein.
[b] Classification of results is given in parentheses when not negative.

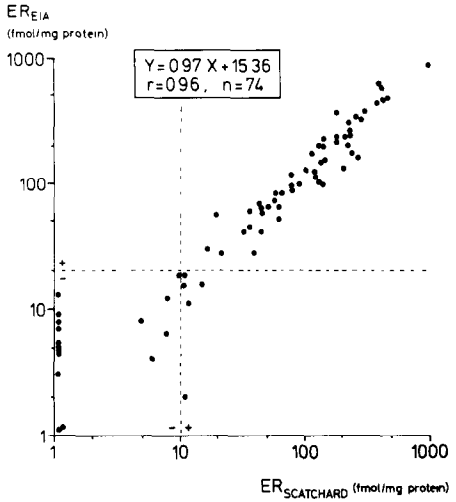

Fig. 1. Relationship between results of the enzyme immunoassay (EIA) and the ligand binding assay (Scatchard) for the estimation of oestrogen receptors (ER) in human breast cancer cytosol.

with the results obtained with the ER-EIA. From this table it appears that also with the enzyme immunoassay, the majority of human meningioma cytosols are ER-negative. To further demonstrate the proper performance of the ER-EIA, 74 human breast cancer cytosols were assayed by both methods. The result in Fig. 1 shows that a good agreement between ligand binding and enzyme immunoassay was observed.

To exclude the possibility that meningioma's are ER-negative because of metabolic degradation of either ligand or a putative ER, six meningioma cytosols were mixed equally with ER-positive human myometrium cytosol and reassayed by Scatchard plot analysis. All mixtures were ER-positive, and the ER level was $53 \pm 6\%$ (mean $\pm$ SD) of that of the uterus cytosol. A typical example of such an experiment is shown in Fig. 2. These results show that the occurrence of metabolic

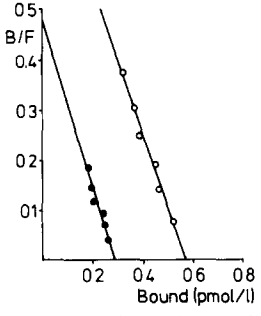

Fig. 2. Scatchard plots of the binding of oestradiol to a human myometrium cytosol (○———○) and to a 1 : 1 mixture of this cytosol with an oestrogen receptor-negative human meningioma cytosol (●———●).

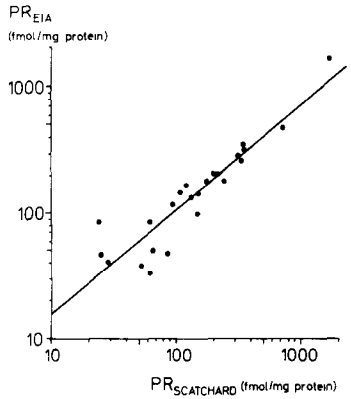

Fig. 3. Relationship between results of the enzyme immunoassay (EIA) and the ligand binding assay (Scatchard) for the estimation of progestin receptors (PR) in human meningioma cytosol. After logarithmic transformation of the results the regression line was $y = 0.83x + 0.36$; $r = 0.9171$; $n = 24$; $p < 0.001$.

degradation of either ligand or receptor is very unlikely to be the reason for the absence of ER from meningioma cytosol.

A highly significant correlation was observed between the results of the EIA and the Scatchard analysis for PR in freshly prepared human breast cancer cytosols ($r = 0.953$; $n = 19$). Moreover, such a correlation was also observed when human meningioma cytosols were assayed by both methods (Fig. 3). This means that antibodies against the human progestin receptor recognize the progestin receptor in meningioma tissue and thus provides an additional indication that the meningioma progestin receptor is a true receptor. Five non-meningioma intracranial tumour tissues, i.e. one pituitary tumour and 4 gliomas, were also analysed for the presence of PR by both methods. None of these samples was positive.

Discussion

The data reported here indicate that monoclonal antibody based enzyme immunoassays can be useful tools for the study of oestrogen and progestin receptors in cytosols of steroid target tissues.

The present study once more demonstrates that the majority of human meningioma tissues is devoid of oestrogen receptors and that the synthesis of progestin receptors in this tissue appears to be independent of the presence of oestrogen receptors. The absence of ER from meningioma cytosols is not caused by metabolic degradation of the ligand or the receptor, because only a 50% reduction occurred in the ER level of human myometrium cytosol after a 1 : 1 dilution with an ER-negative meningioma cytosol. If the latter contained a steroid metabolizing enzyme or a protease capable of degrading oestrogen receptors, a reduction much larger than 50% would have been observed. Other experiments were carried out, in which tritiated oestradiol was incubated with meningioma cytosol and the incubation

mixture was analysed by thin layer chromatography for the occurrence of oestradiol metabolites. These experiments demonstrated that under the conditions used for ER assay, i.e. an overnight incubation at 4 °C, no metabolism of the ligand occurred (unpubl. obs.).

Our observation that PR in human meningioma cytosol can be assayed equally well by EIA as by Scatchard plot analysis is a very important one. It indicates that the progestin receptor hitherto detected in meningioma by radioligand binding assays, is recognized by monoclonal antibodies against bona fide human progestin receptors. This is a further indication that the progestin binder in meningioma is very likely a true progestin receptor and emphasizes the need to fully establish the mechanism, if any, by which progestins and antiprogestins may influence the growth of meningiomas.

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