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ENZYME IMMUNOASSAY OF PROGESTERONE RECEPTOR IN BREAST CANCER BIOPSY SAMPLES

A comparison with the dextran coated charcoal method

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Abstract

Progesterone receptor was measured in 97 breast cancer biopsy samples with two methods, the multiple point dextran coated charcoal method with Scatchard analysis and enzyme immunoassay. The measurable amount of progesterone receptor was usually higher with enzyme immunoassay (mean increase 30%). When comparing the two techniques quantitatively a high correlation coefficient was obtained (r_s=0.88; $p<0.001$). A concordance in terms of progesterone receptor positivity and negativity was obtained in 81 of the samples (84%) when using 10 fmol/ml as the borderline value. If instead the cut-off value used for clinical evaluations at our laboratory (30 fmol PgR/mg protein) was applied, the concordance increased to 94%. The lack of concordance in the remaining samples may be due to difficulties in the interpretation of the Scatchard plots, especially at low receptor concentration levels and to differences in specificity between the two methods in certain samples. One obvious advantage with enzyme immunoassay compared to the dextran coated charcoal method is that significantly less tissue is needed.

Key words: Breast cancer, progesterone receptor, surgical biopsies, enzyme immunoassay, dextran coated charcoal method.

Estrogen (ER) and progesterone receptors (PgR) are usually measured routinely in breast cancer samples, mainly due to their importance in the selection of systemic treatment (cytotoxic or endocrine) for metastatic breast cancer. Hitherto, the receptor measurements have usually been performed with techniques attempting to measure the binding of radio-labelled ligands to their specific receptors. Examples of such techniques are the multiple point dextran coated charcoal method with Scatchard analysis (DCC), sucrose density gradient centrifugation and isoelectric focusing. In addition, an enzyme immunoassay (EIA) for ER measurement has shown good concordance with the radioligand methods. As EIA is a rapid and simple technique and requires only small amounts of tissue, EIA has been suggested to be a practical method for ER measurement in surgical biopsies (1) and fine needle aspirates (2) from breast cancers. Recently a similar method for PgR has been developed (PgR-EIA;4), presumably having the same advantages as ER-EIA. In the present investigation EIA was therefore compared with DCC (our routine technique) for PgR measurement in breast cancer biopsy samples.

Material and Methods

Tissue material. The comparison between DCC and EIA for the measurement of PgR was carried out in 97 consecutive breast cancer biopsy samples. Twenty samples derived from premenopausal patients (less than one year after the last regular menstrual bleeding) and 77 from postmenopausal patients (more than one year after the last regular menstrual bleeding). The biopsy samples were obtained at operation and then kept for not more than 2 weeks at either −70°C or in liquid nitrogen until analysis.

Chemicals and ligands. All chemicals were of analytical grade. ^{3}H-promegestone (^{3}H-R5020, 3.2 TBg/mmol) and unlabelled promegestone were purchased from New England Nuclear (Boston MA, USA). The PgR-EIA kits were kindly supplied by Abbott Laboratories, Diagnostic Division, North Chicago, Ill., USA.

Analytical procedure. Tumour tissues were homogenized at 0°C with an all-glass Potter-Elvehjelm homogenizer in TE buffer (Tris-HCl 10 mmol/l, EDTA 1.5 mmol/l, pH 7.4 at 20°C), whereafter the samples were centrifuged at 20000 × g for 10 min at 0°C. The protein

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concentration in the supernatant was adjusted to 1–3 mg/ml ($OD_{280-310}$), whereafter the PgR concentration was determined with our routine DCC method and EIA as described below.

To the aliquots for DCC assay, mercaptoethanol and glycerol were added to final concentrations of 10 mmol/l and 10% (v/v) respectively. The PgR measurement was then performed as described previously with an incubation time of 2 h. The level of senstivity was 10 fmol/ml supernatant (3). Values above or equal to this level were considered to be PgR positive (PgR+) and those below as PgR negative (PgR−). To the aliquots for EIA, monothioglycerol and sodium molybdate were added to final concentrations of 1.0 mmol/l and 5.0 mmol/l respectively. The assay was thereafter performed according to kit instructions with an incubation time of 18 h (Abbott Laboratories, USA). The level of sensitivity for PgR-EIA is approximately 1.0 fmol/ml, but to get a more comparable cut-off point with DCC the same border-line value (10 fmol/ml) was chosen to distinguish between PgR+ and PgR−. As a comparison the cut-off value used for clinical evaluations at our laboratory was applied for both methods (30 fmol/mg protein).

Statistics. Correlations were estimated according to Spearman's rank-correlation (r_s) and p-values below 0.05 were considered as indicating statistical significance.

Results

Out of 97 samples, 52 were positive and 29 negative with both techniques (Table 1, cut-off value 10 fmol/ml). This gives a concordance in terms of positivity and negativity of 84%. From Tables 1 and 2 can also be seen that 13 of the 16 samples showing discordance were PgR+ with EIA (range 11–180 fmol/ml) but PgR− with DCC and all from postmenopausal patients, whereas the remaining 3 samples were PgR− with EIA but PgR+ with DCC (range 22–82 fmol/ml) and all from premenopausal patients. When using the clinical cut-off value instead (30 fmol/mg protein), the concordance increased to 91 out of the 97 samples (94%).

When comparing the 2 techniques quantitatively a r_s-value of 0.88 ($p<0.001$) was obtained (Figure). As can be seen from the figure the correlation was closer at higher PgR levels. The r_s-value for samples with PgR_{EIA} values below 100 fmol/ml was 0.40 and for samples above or equal to 100 fmol/ml it was 0.91. The PgR recovery was increased by a mean factor (±SD) of 1.3±0.66 for the 52 samples which were positive by both techniques when EIA was used instead of DCC. This factor was 1.2±0.62 (n=14) for samples from premenopausal patients and 1.4±0.69 (n=38) for samples from postmentopausals (NS). In agreement with this mean increase in the measurable amount of PgR with EIA, most samples (n=48) also had higher concentration estimates with EIA than with DCC. For 20 samples, however, higher PgR values was

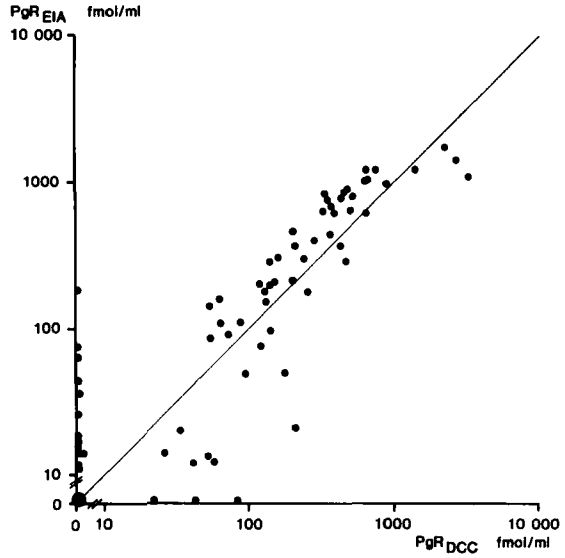

Figure. Comparison of PgR recovery (fmol/ml) in 97 supernatant samples from human breast cancer measured with DCC and EIA. The 45°C slope is indicated in the figure. $r_s=0.88$, $p<0.001$.

Table 1

PgR status (cut-off value 10 fmol/ml) for samples measured with both EIA and DCC. The number of samples from pre- and postmenopausal (pre/post) patients are shown in parenthesis

	DCC	
	PgR+	PgR−
PgR+	52 (14/38)	13 (0/13)
PgR−	3 (3/0)	29 (3/26)

obtained with the DCC technique. The latter finding was most pronounced for samples with PgR_{EIA} values below 100 fmol/ml.

Discussion

Enzyme immunoassay is a recently developed technique for the measurement of PgR (4). It is based on direct antigenic recognition of PgR by monoclonal antibodies. Hitherto, the measurement of PgR has been performed in our laboratory with the dextran coated charcoal method and Scatchard analysis (DCC). The main disadvantages with this method according to our experiences (about 4000 analyses of PgR in breast cancer samples) are interpretation difficulties of the Scatchard plots, especially at low PgR concentrations, and the need for a considerable amount of tissue. EIA has been shown to be a practical method for ER measurement which only requires small amounts of tissue. It was therefore of great interest to compare EIA estimation of PgR with our DCC method.

In agreement with a previous report (4), the present

Table 2

Concentrations (fmol/ml) of PgR (measured with DCC and EIA) and ER (measured with isoelectric focusing (IF)). Kd-value (DCC method), age and menopausal status for the 16 samples showing disconcordance in PgR negativity/positivity between DCC and EIA

Sample No	Age	Menopausal status	PgR_{DCC}	PgR_{EIA}	ER_{IF}	Kd $\times 10^{-10}$
1	79	POST	0	11	820	–
2	79	POST	0	12	280	–
3	56	POST	0	14	58	–
4	56	POST	0	14	170	–
5	82	POST	0	16	14	–
6	85	POST	0	17	0	–
7	56	POST	0	18	92	–
8	79	POST	0	26	210	–
9	86	POST	0	36	7.6	–
10	82	POST	0	44	64	–
11	53	POST	0	63	1 300	–
12	76	POST	0	75	370	–
13	78	POST	0	180	0	–
14	53	PRE	22	0	7.1	5.9
15	43	PRE	43	0	0	9.8
16	48	PRE	82	0	29	4.4

investigation has shown a highly significant correlation between DCC and EIA for PgR measurement in breast cancer samples. When comparing the 2 methods in terms of PgR positivity and negativity, concordance was obtained in 84% of the samples. The discrepancy in the remaining samples may in part be explained by PgR concentrations near the level of method sensitivity, which at least for the DCC method causes interpretation difficulties for the Scatchard plots. Thus, 7 of the 13 samples being PgR+ with EIA but PgR− with DCC had PgR_{EIA} concentrations between 10–20 fmol/ml (Table 2). Also 2 of the 3 samples being PgR+ with DCC but PgR− with EIA gave Scatchard plots which were difficult to evaluate. If instead the cut-off value used for clinical evaluations at our laboratory (30 fmol PgR/mg protein) was applied the concordance increased to 91 out of 97 samples (94%). But still some samples remained which were negative with one technique but clearly positive with the other. This finding is in disagreement with the results obtained when EIA and isoelectric focusing were compared for ER measurement (1). The discordance between EIA and DCC for PgR assay may in some cases be due to the choice of radioligand and differences in binding specificity. ^{3}H-Org-2058 has been recommended by the EORTC receptor group instead of ^{3}H-R5020 (used in this study) for PgR measurement (5). In a pilot study at our laboratory an overall good agreement between the 2 ligands was obtained (r_s=0.91, n=19), but in one sample, the PgR concentration was considerably higher when measured with ^{3}H-R5020 (850 fmol/ml) than with ^{3}H-Org-2058 (59 fmol/ml). It should furthermore be mentioned that all samples which were PgR+ with EIA but PgR− with DCC derived from postmenopausal patients, whereas the 3 samples showing the opposite pattern all derived from premenopausal patients. An explanation for this observation has not yet been found. When comparing with ER content measured with isoelectric focusing in the same samples, the samples which were PgR_{EIA}+ but PgR_{DCC}− usually (10 out of 13) were ER+ (≥10 fmol/ml), whereas 2 out of 3 samples with the opposite pattern were ER−. As presence of PgR is closely related to presence of ER, these findings suggest that the results obtained with EIA are more reliable.

When the absolute PgR concentration values were compared 30% higher values were obtained with EIA than with DCC. Forty-nine samples had higher values with EIA, whereas the opposite occurred in 20 samples. These differences could not be explained by menopausal status, concentration level or by the dissociation constant (Kd) obtained from the Scatchard plot. It should be noted that the EIA cytosol contained molybdate, whereas the DCC cytosol did not. Molybdate has been reported to stabilize receptor and give higher PgR values (6). In control experiments we were, however, unable to demonstrate any significant increase of the measurable amount of PgR with DCC when molybdate was added to the buffer (n=10; concentration range 0–1 800 fmol/ml; mean increase ±SD 3.4±12%). It should also be mentioned that a shorter incubation time was used with DCC than with EIA (2 versus 18 h). Although an overall satisfactory concordance between 2 and 18 incubation time previously has been obtained with DCC at our laboratory (data not shown), one cannot exclude that the discrepancy between DDC and EIA in certain samples in the present study may have been caused by the difference in incubation time.

In conclusion, this investigation showed an overall good concordance between DCC and EIA for the measurement of PgR. This finding, and also the fact that EIA is simple, rapid, and needs only small amounts of tissue, supports the suggestion that EIA may be a practical method for PgR measurements in breast cancer biopsy samples. The discrepancies between EIA and DCC in certain samples may be due to interpretation difficulties with the Scatchard plots in the DCC method or to differences in specificity, which should be further investigated.

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REFERENCES

1. Fernö M, Borg Å, Sellberg G. Enzyme immuno assay of the estrogen receptor in breast cancer biopsy samples. A comparison with isoelectric focusing. Acta Radiol Oncol 1986; 25: 171.
2. Borg Å, Fernö M, Idvall I. Estrogen receptor enzyme immuno-assay in fine needle aspirates. Acta Oncol 1989; 28 (Accepted for publication).
3. Norgren A, Borg Å, Fernö M, Johansson U, Lindahl B, Tsiobanelis K. Improved method for assay of estradiol and progesterone receptors with special reference to breast cancer. Anticancer Res 1982; 2: 315.
4. Weigand R, Cotter D, Przywara L, Nolan C. Measurement of human progesterone receptor (PgR) by the double monoclonal enzyme immunoassay, PgR-EIA monoclonal. Meeting Abstract 103. International Society for Oncodevelopmental Biology and Medicine, XV Annual Meeting, Quebec Canada, 1987.
5. Koenders A, Thorpe SM. Standardization of steroid receptor assays in human breast cancer. I. Reproducibility of estradiol and progesterone receptor assays. Eur J Cancer Clin Oncol 1983; 19: 1221.
6. Andersson KM, Phelan J, Marogil M, Hendrickson C, Economou S. Sodium molybdate increases the amount of progesterone and estrogen receptor detected in certain human breast cancer cytosols. Steroids 1980; 35: 273.