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Prolactin Receptors in Human Breast Cancer

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Abstract—*Prolactin receptors have been measured in 92 human breast carcinomas. Both free and total receptors (after desaturation by $MgCl_2$) have been looked for. Free receptors have been found in 46% of the cases, total receptors in 72%. Specific binding ranges from 0.8 to 8.0%. No correlation could be found between prolactin receptors and age, weight, menopausal status and pathological features (differentiation, histoprognostic grading, cellular density). A highly significant correlation has been found between prolactin receptors on the one hand and estradiol and progesterone receptors on the other.*

INTRODUCTION

THE INVOLVEMENT of prolactin in mammary tumors is well-established in rodents. Prolactin stimulates the appearance and growth of spontaneous or DMBA-induced tumors in rats; in mice, prolactin only induces preneoplastic lesions and has no effect on the growth of the adenocarcinomas [1]. The role of prolactin in human breast cancer is not established. Many detailed reviews have been devoted to the subject [2–4]. In most studies [5–14], prolactin plasma levels have not been found to be different between cancer patients, or a high-risk population, and control. However Murray *et al.* [15], Rolandi *et al.* [16] and Aldinger *et al.* [17] obtained higher values in breast cancer patients; the prolactin response to TRH was also found to be higher than in controls [18]. Night prolactin plasma levels are lower in post-menopausal patients and higher in pre-menopausal ones [19]. Serum prolactin concentrations were found to be higher in the follicular phase of the menstrual cycle in breast cancer [20]. Kwa and Wand [21] found an abnormal luteal phase evening peak in women with a family history of breast cancer. Breast cancer was found to be more frequent among Rauwolfia derivative users in three studiees [22–24], the relative risk being from 2 to 4. However, most authors have not reported such results [25–32]. Finally, even anti-prolactinic treatments have given disappointing results [31–36].

The discovery of prolactin receptors in breast cancers [37–41], the stimulation of growth of human tumor cells by prolactin [43–45] and the presence of lactalbumin in tumor cells prompted us to look for prolactin receptors as they appeared to be a sign of hormonal sensitivity. A first publication on a heterogeneous group of breast cancer patients (primary and recurring) had shown the interest of the desaturation of receptors before the assay [46].

The example of steroid is encouraging too. Endocrine therapy was widely used prior to knowledge of oestrogen and progesterone receptors. Measurement of these receptors has proved to be of some use in selecting patients for anti-estrogen therapy and in prognosis.

MATERIALS AND METHODS

Patients

The presence of prolactin receptors was looked for in 92 patients undergoing surgery for primary breast cancer in the Centre Oscar Lambret (Lille). Tumor specimens were taken immediately after surgery, fat was removed and samples were divided into two parts: one was submitted for pathological examination, the other immediately frozen and sent for receptor analysis. All the tumors were adenocarcinomas.

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Methods

Tissue processing. The frozen tissue was weighed and then pulverized with a Thermovac Tissue Pulverizer (Thermovac Industries Corp., NY). The tissue was homogenized in Tris 0.02 M, EDTA 3 mM, dithiotreitol 1 mM, azide 0.01%, pH 7.8 buffer.

The homogenate was centrifuged at 800 *g* for 10 min and the supernatant ultracentrifuged at 105,000 *g* for 60 min. The supernatant (cytosol) was carefully removed with a syringe in order to avoid the floating lipid layer. The pellet (microsomal fraction) was re-suspended in 25 mM Tris–HCl. 10 mM $MgCl_2$ buffer, pH 7.8. Later, the protein concentration was determined by the method of Lowry *et al.* [47] applied either directly in the cytosol or after extraction from the membrane (with NaOH 1 N) in the microsomal fraction.

Hormones. Labelling of hGH (NIH HS 2160 E, 20 IU/mg) and hPRL (NPA batch 5 AFP 1582 C) was carried out with Na[^{125}I] using low concentrations of chloramine T [48, 49]. The binding capacity of these hormones was assayed in a fraction enriched on sucrose 2 M with human tumor membranes; hGH was found to have a higher binding capacity and was therefore used as the marker in the assays. GH is a lactogenic hormone in primates [50] and it acts in the same way as hPRL on breast cancer epithelial cells in culture [51]. The specific activity was approximately 75 μCi/g as determined directly or by the self-displacement method [52]. Between 70 and 80% of the iodinated GH added could be specifically bound to prolactin receptors in receptor-enriched membranes (e.g. mammary glands from lactating rabbits pre-treated with CB 154: Sandoz, Basel, Switzerland) [53]. Ovine PRL (NIH-P-S13 30 IU/mg) was used to displace labelled hGH. [^{3}H]-17-β-Estradiol (sp. act. 135 Ci/mmol), [^{3}H]-R 5020 were purchased from N.E.N. (Boston, MA), non-radioactive diethystilboestrol and cortisol were purchased from Steraloids, Inc. (Pawling, NJ).

Assay of prolactin receptors (PRL R). Free receptors. The assay was performed in duplicate according to Shiu [54]. Four hundred micrograms of membrane proteins were incubated with approximately 100,000 counts/min of iodinated GH in the presence or absence of a 1000-fold excess of unlabelled oPRL (1 μg). The final incubation volume was adjusted to 0.5 ml with Tris–$MgCl_2$ buffer (pH 7.6) containing 0.1% bovine serum albumin.

Total receptors. Since prolactin does not appear to dissociate from its receptors during membrane preparation, de-saturation of occupied receptors was performed before the assay of prolactin sites [49]. To accomplish this, crude membrane proteins normally used in the assay were pre-incubated with 0.5 ml of 3 M $MgCl_2$ for 5 min and 4 ml of cold Tris–HCl buffer (pH 7.6) containing 0.1% BSA were then added to each tube. Following centrifugation at 2200 *g*, the pellets were re-suspended in Tris–HCl buffer and binding of the labelled hormone was assayed as described above.

Specific binding was calculated as the difference between the radioactivity bound in the absence and the presence of an excess of unlabelled hormone and expressed as the percentage of the total radioactivity added.

Assay of estradiol receptors (ER) and progesterone receptors (PgR). ER was determined by the DCC method [55]. Scatchard analysis of binding data was performed in order to quantify the number of free binding sites per mg of cytosol protein. Non-specific binding was estimated from incubations with an excess of unlabelled DES, which avoids interference with TeBG. PgR were also assayed by the DCC method [56], using tritiated and non-radioactive R 5020.

Statistical analysis

The relation between quantitative variables was studied by the calculation of the Spearman rank correlation coefficient *r*.

The relation between qualitative variables was studied by analyses of variance. Results were calculated according to Fischer–Snedecor's *f*-value.

RESULTS

Specific binding of iodinated hGH

The specific binding of [^{125}I]-labelled hGH to plastic tubes, i.e. to 'non-physiological' binding sites, was estimated by measuring the apparent displacement of [^{125}I]-hGH per μg of unlabelled oPRL in the absence of tumor membranes or in the presence of 400 μg of denatured boiled membranes. Its maximal value was 0.8% of the total radioactivity added. Therefore tumors whose specific binding was greater than 0.8% were considered positive (PRL R_+). Total binding averaged between 5 and 13%, whereas non-specific binding ranges between 5 and 9% of the total radioactivity added.

Forty-six per cent of the tumors were positive when free receptors were measured, while 72% were positive in terms of total receptors. The mean values of the specific binding were respectively $1.69 \pm 0.9\%$ for free receptors and $2.43 \pm 1.65\%$ for total receptors. Treatment with $MgCl_2$ led to an approximate 30% loss of

tumor membrane proteins [48]. In spite of this, $MgCl_2$ treatment led to an increase in prolactin binding in 80% of the tumors. It allowed positivation in free prolactin receptor of receptor-negative tumors; in one case, the specific binding was increased five-fold.

The specific binding activity ranged between 0.8 and 5.6% for free receptors, and between 0.8 and 8.0% for total receptors. In most cases it was between 0.8 and 4.6% (Fig. 1).

Relations with clinical features

In our population a correlation has been found between estrogen receptor and age ($f = 3.995$) and histoprognostic grading according to Scarff and Bloom ($f = 3.256$), and between progesterone receptor and weight ($r = 0.299$) and histoprognostic grading ($f = 3.256$). Prior treatment by chemotherapy had no effect on the level of estrogen, progesterone and prolactin receptors.

A correlation was looked for between prolactin receptors and age, weight, menopausal status, pathological differentiation, histoprognostic grading, cellular density, stromal reaction and axillary metastasis. None could be found.

Thus if prolactin and estrogen receptors are linked, it is improbable that it is through these factors.

Relations with hormonal features

Prolactin levels were assayed in all the patients just before surgery. No relation was found between prolactin plasma levels and prolactin receptor levels.

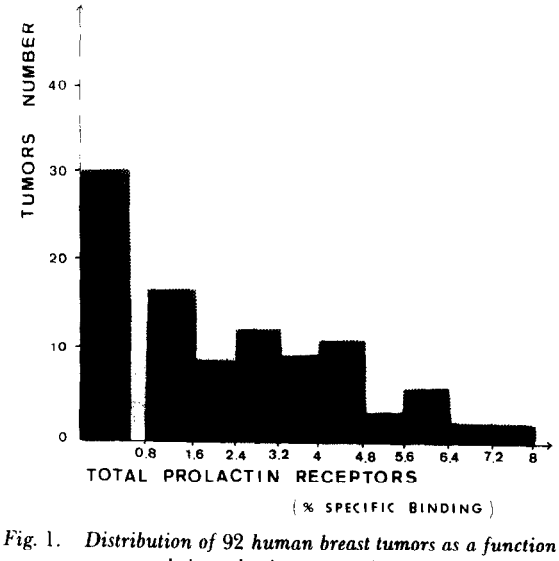

Fig. 1. Distribution of 92 human breast tumors as a function of their prolactin receptor levels.

No correlation could be found between prolactin receptors on the one hand and estradiol or estrone plasma levels on the other.

A highly significant correlation was found between saturated prolactin and estrogen receptors ($r = 0.24$) on the one hand and progesterone receptors on the other ($r = 0.225$). No correlation was found, however, between total prolactin receptors and estradiol or progesterone receptors. When the positivity threshold was 3 fmol/mg of cytosolic proteins (the limit of the biochemical detection), there was no relation between the presence of RE and the presence of free prolactin receptors ($\chi_2 = 3.05$).

When the positivity threshold of RE was 50 fmol/mg of cytosolic protein (the limit for hormonotherapy in the Centre Oscar Lambret), the correlation between RE and free PRL R was closely linked ($\chi^2 = 5.23$) (Table 1).

DISCUSSION

Our results confirm those already obtained by other authors [37–42] (Table 2). Prolactin receptors exist in some human breast cancers. The method used is very close in every study. The most important difference is the iodinated hormone used: generally human prolactin [37, 38, 41, 42], but ovine prolactin too, [39, 41, 42] and human GH [38, 42, present study]. Turcot-Lemay and Kelly [42] have shown that the percentage of prolactin receptor-positive tumors varies from 12% with hGH to 30% with ovine prolactin and 58% with human prolactin. The author concludes that human prolactin is the best hormone for the assay. To our knowledge, as yet no other author has tried to de-saturate the receptors in breast cancer, but it has been done using other tissues; we have seen that it appears physiologically logical and gives better results in

Table 1. Number of tumors with prolactin receptors as a function of ER threshold

ER (fmol/mg protein)	No. of tumors	Free PRL R.
(1) < 3	11	3
(2) > 3	64	30
(3) < 50	34	11
(4) > 50	41	22

The presence of free PRL R is linked to the presence of ER when ER > 50 fmol/mg cytosolic protein.

Table 2. Comparison of the results of prolactin receptor assays published

References	No. of tumors	Iodinated hormone	% Positive tumors	Range of % specific binding
[37]	41	hPR	20	1–4.2
[38]	20	hPR	70	0.5–2.5
		hGH		0.5–5.1
[39]	111	oPR	51	1–9.22
[41]	83	hPR	32.5	1–4.3
[40]	8	oPR	37.5	
[42]	759	hPR	58	
		oPR	30	0.5–> 10
		hGH	12	
Present study	92	hGH	free 46 total 72	0.8–8

specific binding. Nevertheless, the average value of the percentage of prolactin receptor-positive tumours is around 50%. The range of specific binding is very close in all the studies, the maximum value being around 10% [42].

To our knowledge, it is the first time that a statistically significant correlation has been found between prolactin receptors and steroid receptors. It is interesting to note that in DMBA-induced tumors in rats it has been shown that prolactin and estrogen induce prolactin and estrogen receptors, estrogen inducing progesterone receptors too [57]. All these receptors are thus inter-linked.

No correlation has been found between RE and RPg on the one hand and total receptors on the other. $MgCl_2$ acts on internalized receptors, which seem not to have any physiological role [58]. Contrary to estrogen receptors, no correlation could be found between prolactin receptors and clinical or pathological features. This could be explained by the fact that estrogen is a major hormone for epithelial differentiation and growth, while prolactin can act only on growth [59]. The inter-relations between the receptors do not exist through pathological features.

Prolactin receptors are much more frequent in estrogen- and/or progesterone-positive tumors. That is to say, among estrogen-positive and progesterone-positive tumors prolactin receptors select a population of particularly hormone-sensitive cells.

It is important to note that some steroid receptor-negative tumors have prolactin receptors (3 out of 11 of our patients). It might be clinically useful in those cases whose prognosis is bad to propose an anti-prolactinic treatment. Its precise modalities must be studied; it has been shown [60, 61] that hypophysectomy (by surgery or yttrium) leaves enough prolactin-secreting cells to allow secretion which is either spontaneous or follows TRH stimulation in a high percentage of cases. This treatment should be associated with medical anti-prolactinic treatment [61].

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