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Effect of Bromocriptin Treatment on Prolactin and Steroid Receptor Levels in Human Breast Cancer

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Abstract—*We have investigated the effect of bromocriptin, a prolactin-lowering drug, on prolactin, estradiol and progesterone receptors in breast cancers. Doses of* 2.5 mg per os *of bromocriptin were given twice daily for* 4 days *before surgery. The efficiency of the treatment was evaluated by assaying the plasma prolactin level. The results obtained for the treated population* (n = 30) *was compared to those obtained for an untreated one* (n = 120) *during the same period. Bromocriptin does not increase the available prolactin receptors in breast cancer specimens. Unmasking* in vitro *receptors with* $MgCl_2$ 3 M *leads in both cases to an increase in prolactin receptors. The number of positive estradiol receptor tumors was increased after bromocriptin treatment. Neither the rate of positivity nor the level of progesterone receptors is changed by the treatment. This study demonstrates clearly the inefficiency of bromocriptin in unmasking* in vivo *prolactin receptors in breast cancer.*

INTRODUCTION

PROLACTIN (PRL) is an important hormone controlling the growth of the normal mammary gland and the synthesis activity of the normal mammary cell [1, 2]. These actions are mediated by specific membrane receptors [3, 4].

PRL is involved in induction and promotion of experimental mammary cancers [5, 6]. Prolactin receptors (PRL R) have been detected and characterized in 7,12-dimethylbenz(a)anthracene (DMBA)- and nitrosomethylurea (NMU)-induced mammary tumors in rats [7, 8].

The role of PRL in human breast cancer has not yet been established [9]. Results are controversial but there are several lines of evidence suggesting that prolactin may play a role in induction and/or promotion of breast cancer. We and others have demonstrated that PRL binding is detectable in 50% of the tumors [10–19]. These binding sites have all the characteristics of receptors [20]. Nevertheless the levels of prolactin receptors in breast cancer are low and difficult to detect. We previously postulated that, since specimens are obtained in surgery, the rise in prolactin plasma levels during general anesthesia [21, 22] may lead to the blocking of available receptor sites. For this reason, in a previous work [15] we treated the breast cancer cell membranes with 3 M $MgCl_2$ *in vitro* to desaturate the receptors: the percentage of PRL R-positive tumors and the levels of PRL R were both increased when compared to the conventional PRL R assay.

In the present work we titrated the amount of prolactin receptors, avoiding their occupancy by endogenous prolactin *in vivo*. This was achieved by using bromocriptin, a compound which is known to depress prolactin secretion.

MATERIALS AND METHODS

Patients and treatment

All the women taking part in this study were seen at the Centre Oscar Lambret (an anticancer center in the north of France). Tumor specimens

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were obtained from women undergoing surgery for primary breast cancer (adenocarcinomas >2 cm) with no evidence of metastatic or inflammatory disease. Between January 1983 and September 1983 30/150 were randomly selected for treatment by bromocriptin (CB 154, Parlodel, Sandoz). There is no difference between the histological characteristics of the treated group and controls as regards tumor type (Table 1), cellular density, differentiation and histological grading.

Doses of 2.5 mg of bromocriptin were given *per os* twice daily for 4 days before surgery. The last dose was administered immediately before surgery. The efficiency of the treatment was evaluated by assaying the plasma PRL level (<10 ng/ml). Ten milliliters of peripheral blood were collected in dry tubes during anesthesia. Hormone levels were measured using a commercial kit: PRL (Abbott).

Collection of tumors and tissue processing

At the time of surgery fat was removed and the samples were divided into three pieces: one was submitted for histologic studies and the others were frozen until receptor determinations were carried out.

The frozen tissues were weighed and then pulverized (Spex freezer/Mill, Bioblock, France). The tissue was homogenized in Tris 0.02 M, EDTA 3 mM, dithiothreitol 1 mM, azide 0.01%, pH 7.6 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 resuspended in 25 mM Tris–HCl, 10 mM $MgCl_2$, pH 7.6 buffer. The protein concentration was determined by the method of Lowry *et al.* [23] either directly in the cytosol or after extraction from the membrane with NaOH 1 M in the microsomal fraction.

Hormones

Labeling of hPRL (NPA batch 5 AFP 1582 C) was carried out with Na[^{125}I] using a low concentration of chloramin T [24, 25]. [^{125}I]hPRL was then purified on rabbit mammary gland membrane receptors as described elsewhere [20]. The specific activity was 75 μCi/μg. Ovine PRL (NIH-P-S-15 30.5 IU/mg) was used to displace labeled hPRL. [^{3}H]17β-Estradiol (135 Ci/mmol) and [^{3}H]promegestone (R_{5020}) were purchased from New England Nuclear (Boston, MA). Non-radioactive steroids were purchased from Steraloids (Pawling, NJ).

Assay of PRL R

Four hundred micrograms of membrane proteins were incubated with approximately 200,000 counts/min of iodinated hPRL in the presence or absence of a 1000-fold excess of unlabeled oPRL (1 μg) as described elsewhere [15, 17]. The final incubation volume was adjusted to 0.5 ml with Tris–$MgCl_2$ buffer (pH 7.6) containing 0.1% bovine serum albumin (free PRL R). Since PRL does not appear to dissociate from its receptors during membrane preparation, when it was possible desaturation of occupied receptors with $MgCl_2$ 3 M was performed before the assay of PRL sites [15] (total PRL R).

Assay of estradiol receptors (ER) and progesterone receptors (PgR)

Steroid receptors were determined by the DCC method [26]. The ER content of the diluted cytosol was determined by incubation of 100 μl cytosol (in duplicate) with 50 μl of four doses (10, 5, 1 and 0.5 mM) of [^{3}H]2,4,6,7-estradiol with and without a 100-fold excess of diethylstilbestrol (DES). PR was assayed with four doses (10, 5, 1 and 0.5 mM) of [^{3}H]R_{5020} with and without a 100-fold excess of non-labeled R_{5020}.

The radioactive ligands and cytosol were incubated at 4°C for 16 hr. A 500-μl suspension of dextran-coated charcoal (DCC) (2,5 mg Norit A-activated charcoal, 250 μg dextran in 1 ml Tris buffer, pH 8.0, 4°C) was added to each tube. Tubes were then shaken for 45 min at 0–4°C and then sedimented by centrifugation at 4°C for 20 min at 3000 rev/min. The DCC-treated supernatant was combined with 3 ml of Beckman Ready-Solv scintillator and counted in a Beckman Model LS 6800 liquid scintillation counter. The ER and PR data were analyzed by the method of Scatchard to determine the dissociation constants (K_d) and concentrations of ER and PR in each tumor specimen, expressed as fmol of receptor/mg

Table 1. *Histological characteristics of the two populations*

	Without treatment (n = 120)	Bromocriptin treatment (n = 30)
Invasive ductal carcinomas	87 (72.5%)	24 (80%)
Invasive lobular carcinomas	14 (11.7%)	2 (6.6%)
Invasive comedocarcinomas	9 (7.5%)	2 (6.6%)
Others	10 (8.3%)	2 (6.6%)

cytosol protein. The control of ER and PR assays was performed by the EORTC Receptor Group quality control system.

Statistical analysis

The distributions were normal except for ER+ and PgR+ (log normal). The comparison between the two percentages was carried out using the χ^2 method with the Yates correction for small samples and the comparison between the means using Student's method.

RESULTS

PRL levels

Six patients supposed to have received bromocriptin treatment were excluded from the study since their levels of PRL were very high (115, 47, 160, 80, 39 and 20 ng/ml). The PRL mean plasma level was 2.45 ± 0.39 S.E.M. ($n = 30$) (Fig. 1).

PRL R levels

Figure 1 presents the results obtained for PRL R in breast cancers after bromocriptin treatment compared with those of the untreated population. There is no significant difference between the two populations.

There were 49% of free PRL R-positive tumors in the untreated population and 52% in the treated one. In both cases $MgCl_2$ treatment led to an increase in PRL R-positive tumors (80% in the untreated population and 71% in the treated one) and in PRL R levels. Surprisingly there is a slight but not statistically significant decrease in PRL R levels after bromocriptin treatment.

ER and PgR levels

Figures 2 and 3 show the effect of bromocriptin treatment on steroid receptors in breast cancers.

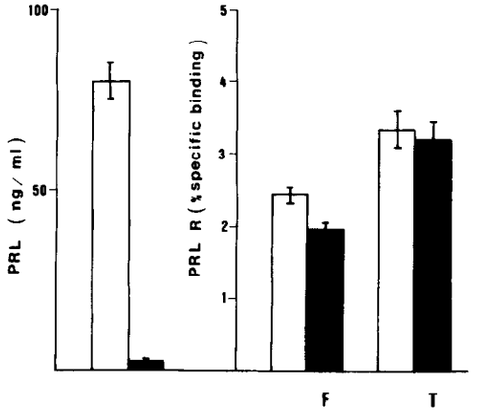

Fig. 1. Left panel: PRL mean level (± S.E.M.) at the time of mastectomy in patients □ untreated (n = 120) *or ■ treated* (n = 30) *by bromocriptin. Right panel: Free (F) PRL R and total (T) PRL R mean levels (± S.E.M.) in positive PRL R breast cancer of patients □ untreated or ■ treated by bromocriptin.*

The ER and PgR were considered positive when greater than 10 and 25 fmol/mg cytosolic protein respectively. The number of ER-positive tumors (Fig. 2) was significantly increased after treatment with bromocriptin in premenopausal women; there was a slight but not statistically significant increase in ER+ mean levels. Neither the positivity nor the mean level of PgR was changed by the treatment (Fig. 3). There was no modification of the statistical significance of the results if we analyzed ER and PR when PRL R were present.

DISCUSSION

The present study shows that bromocriptin does not increase the available prolactin receptors

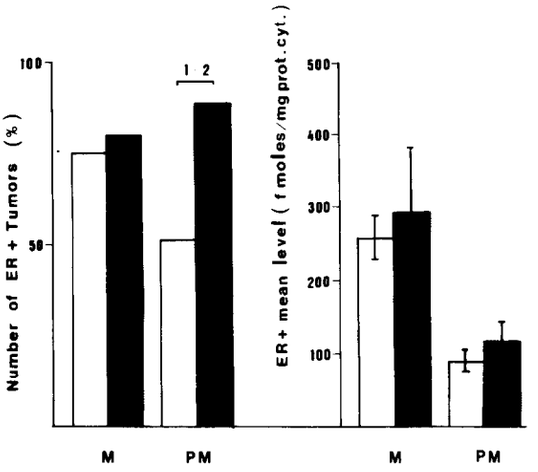

Fig. 2. Left panel: percentage of ER-positive tumors from patients ■ treated or □ not treated by bromocriptin. 1: P = 0.04 (χ^2); 2: P = 0.10 (χ^2 *with Yates correction*). *Right panel: mean levels of ER (± S.E.M.) in positive tumors. M: menopausal patients (untreated,* n = 77; *treated,* n = 21); *PM: premenopausal patients (untreated,* n = 33; *treated,* n = 9).

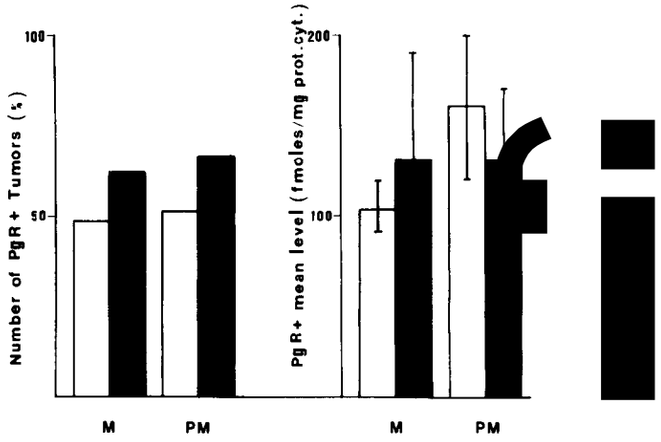

Fig. 3. Left panel: percentage of PR-positive tumors from patients ■ treated or □ not treated by bromocriptin. Right panel: mean levels of PgR (± S.E.M.) in positive tumors. M: menopausal patients (untreated, n = 77; *treated,* n = 21); *PM: premenopausal patients (untreated,* n = 33; *treated,* n = 9).

in breast cancer specimens. High levels of PRL are able to induce a short-term reduction (occupancy) and down-regulation of its own receptors, in normal mammary glands, both *in vivo* [27] and *in vitro* [2, 28]. It has previously been shown that prolactin can also induce a down-regulation of its receptor levels in cultured cells from DMBA-induced tumors in rats [29]. In contrast, in NMU-induced mammary tumor explants down-regulation occurs only at non-physiological levels of PRL (20 μg/ml) [30] and markedly supra-physiological concentrations of insulin are required to down-regulate insulin receptors in tumor cell lines compared with normal cells [31]. This suggests a tumor-associated resistance to receptor down-regulation: if the rise in prolactin plasma levels during anesthesia does not induce a down-regulation in PRL R, then attempting to inhibit this reduction with anti-PRL drug is not useful.

Even after bromocriptin treatment $MgCl_2$ 3 M led to an increase in PRL R-positive tumors and in PRL R levels. This suggests that the unmasking of PRL R by $MgCl_2$ in breast cancer [15] could be explained more by its action on cryptic receptors [32] than by the removing of endogenous PRL.

We observed a slight decrease (not statistically significant) in PRL R after bromocriptin treatment. It is well known that prolactin induces a delayed stimulatory effect on the levels of its own receptors [3, 33]. As bromocriptin was not well tolerated by all the patients, half of them had treatment for nearly 1 week. The decrease in PRL during this time might have been long enough to induce a slight decrease in PRL R.

Finally, we observed an increase in the number of ER-positive tumors in breast cancer of premenopausal women. It has been published previously that prolactin increases the ER levels in DMBA-induced tumors [34], in human breast cancer cells in long-term cultures [35] and in normal mouse mammary tissue [36]: PRL causes the conversion of cytoplasmic ER from inactive (4S) to active (8S) forms and elevates nuclear receptor levels. Thus we could postulate that bromocriptin, which blocks PRL production, inhibits ER translocation and increases cytoplasmic ER forms in breast cancers.

The main contribution provided by the present work is a clear demonstration of the inefficiency of bromocriptin in unmasking prolactin receptors in breast cancer. The effect of this drug on ER levels has to be largely confirmed before a short-term bromocriptin treatment should be proposed to improve ER detection in premenopausal women.

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