Estradiol and Progesterone Receptor Levels in Human Breast Adenocarcinoma in Relation to Plasma Estrogen and Progesterone Levels

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ABSTRACT

Plasma estrogen and progesterone levels were determined in 77 premenopausal and 137 menopausal women at the same time that estradiol receptor (ER) and progesterone receptor (PGR) assays were carried out on their breast cancers. The frequency of ER and PGR is approximately the same in premenopausal and postmenopausal women, but the ER content is much higher in postmenopausal women. Although this is usually ascribed to the occupancy of receptors by endogenous estrogen in premenopausal women, our observations suggest that this is unlikely. The higher ER content in postmenopausal women is probably due to the fact that the cyclic progesterone increase in premenopausal women limits estrogen stimulation of ER synthesis.

Our data suggest that the circulating levels of estrogen in postmenopausal women are sufficient to stimulate ER and PGR when ER is functional. In premenopausal women, on the other hand, high levels of circulating progesterone may inhibit PGR, and the absence of PGR in the breast cancers of premenopausal women should be interpreted warily if the plasma level of progesterone is unknown.

INTRODUCTION

Estrogen receptors and PGR’s are hormone regulated in their target organs. In the endometrium estrogens stimulate the synthesis of both estrogen receptors and PGR’s (20, 30), and their level decreases when circulating progesterone increases (3, 4, 6, 22). Similar regulation of steroid receptors in normal mammary epithelium has been recently described (23). In the rat mammary adenocarcinoma, estrogen receptors are stimulated in vivo directly by prolactin and indirectly by estrogens (14, 34) but are inhibited by progesterone. PGR’s appear to be estrogen dependent (11).

Estrogen receptors and PGR’s have been characterized in human breast carcinoma. Their incidence in pre- and postmenopausal cases is not very different, but the ER content differs markedly (5, 12, 13, 28).

The premenopausal period is characterized by cyclic variations in estradiol and progesterone levels related to ovulation, whereas the variations in these hormones are not cyclic postmenopausally and are probably related to adrenal stimulation (2, 24, 27). These observations suggest that the steroid receptor content in breast cancer might be modulated by circulating hormones. In this study we determined the plasma estrogen and progesterone levels in breast cancer patients and analyzed the results in relation to the ER and PGR content of the tumor.

MATERIALS AND METHODS

Specimens of mammary adenocarcinoma from 77 premenopausal women were assayed for ER’s and PGR’s, and plasma estrone, estradiol, and progesterone levels were measured on the day of the biopsy whenever possible. The date of the last menstrual period was recorded. Tumors from 137 postmenopausal patients were studied at the same time. Receptor data and plasma steroid determinations from both series of patients were compared.

Plasma levels of progesterone were determined by radioimmunoassay with [1,2,6,7-3H]progesterone (specific activity, 103 Ci/mmol) and [6,7-3H]estradiol (specific activity, 46 to 55 Ci/mmol) as tracer. Labeled progesterone was obtained from New England Nuclear, Boston, Mass. [1,2-3H]R5020 (specific activity, 51 Ci/mmol) and R5020 were gifts from Dr. J. P. Raynaud (Roussel-Uclaf, Romainville, France). Labeled compounds were checked for radioactivity by paper or thin-layer chromatography. Peripheral blood was drawn the day of the biopsy at 8 a.m. Plasma estrogens were determined by a modification of the technique of Abraham et al. (1, 26). A tracer amount of labeled steroid was added to each sample for evaluation of the recovery, and each individual result was corrected for losses (26). Plasma progesterone extraction was performed with petroleum ether, which allows a mean recovery of 90%. The losses were not corrected for each individual sample, but the differences among successive determinations were not greater than 5%. Final measurements of estrogen and progesterone were made by radioimmunoassay with the use of highly specific antibodies from the Pasteur Institute (Paris, France) or provided by Roussel-Uclaf.

All tumor samples were frozen in liquid nitrogen within 20 min of excision. For ER determination the tissue was processed according to the method previously described (8) except that the tissue was crushed with a Thermovac pulverizer with liquid nitrogen cooling. The homogenization was performed in 10 mM Tris-HCl/1.5 mM EDTA/0.5 mM dithiothreitol buffer, pH 7.4. The homogenate was centrifuged at 105,000 x g for 50 min. The supernatant (cytosol) was collected and contained 1.5 to 4 mg protein per ml as determined by the technique of Lowry et al. (16).
The total unoccupied cytosol estrogen receptors were measured by incubation of identical aliquots of cytosol with increasing concentrations of \([6,7-^3H]\)estradiol from \(10^{-10}\) to \(5 \times 10^{-8}\) M. After 16 hr incubation at 4°C, hormone, unbound or bound with low affinity, was removed by the addition of dextran-coated charcoal (0.5% charcoal/0.05% dextran in 10 mM Tris-HCl/1.5 mM EDTA/0.5 mM dithiothreitol buffer, pH 7.4), incubation for 30 min at 4°C, and centrifugation for 15 min at 2000 \(\times g\). Nonspecific binding was assessed by the addition of diethylstilbestrol at \(10^{-7}\) M final concentration in a double set of samples.

PGR determination was performed by the method described by Horwitz and McGuire (10). In brief, cytosol is prepared in phosphate buffer (5 mM, pH 7.4) containing 10% glycerol. \([^3H]R5020\) was used as the labeled ligand, and R5020 was used for the determination of nonspecific binding. Identical aliquots of cytosol were incubated at different concentrations of labeled steroid from \(0.5 \times 10^{-9}\) to \(10^{-8}\) M. Unlabeled cortisol at a final concentration of \(10^{-6}\) M was added to all samples to saturate the cortisol binding sites. After 3.5 hr at 4°C, unbound or bound hormone with low affinity was removed by the addition of dextran-coated charcoal (0.25% Norit A charcoal/0.025% dextran in phosphate buffer), blended on a Vortex mixer, and immediately centrifuged for 15 min at 2000 \(\times g\) at 4°C. Under these conditions free and occupied cytosol receptors were measured.

For both steroids the number of binding sites (fmol/mg protein) and the dissociation constant were determined with the use of 8 to 10 different concentrations of labeled steroid. They were calculated from the Scatchard plot (31) of the results.

**RESULTS**

As in our previous study (28), we considered as ER positive tumors that had an apparent ER content higher than 8 fmol/mg protein. We considered as PGR positive tumors that contained more than 10 fmol/mg protein, that being the limit of our assay sensitivity.

In both pre- and postmenopausal patients, about 55% of the tumors contained a measurable amount of ER (ER+) (Table 1). With regard to ER tumor content, the mean value was 32 ± 7 (S.E.) fmol/mg protein in premenopausal patients, whereas it was 174 ± 38 fmol/mg protein in postmenopausal patients (Table 2). The difference is significant.

The incidence of tumors containing PGR (PGR+) is similar in the pre- and postmenopausal patients, and PGR is found in about one-half of the ER+ cases. However, unlike ER, the mean PGR concentration is not significantly different before and after the menopause (Table 2).

It could be suggested that the difference in ER content between pre- and postmenopausal tumors is related to the characteristic hormone levels in the blood. Similarly, it could be expected that in premenopausal patients the hormonal variation of the menstrual cycle might induce variations in the content of receptors for estradiol and progesterone. To check these possibilities we measured circulating hormone levels in our patients (both pre- and postmenopausal) on the morning of their operation.

**Premenopausal Patients.** Chart 1 shows the variations of plasma steroids and of receptor distribution throughout the cycle. Plasma estradiol is more elevated between the 10th and the 20th days. The highest individual values of receptors for estradiol are found in tumors operated during the first menstrual decade.

The peak of plasma progesterone takes place at the middle of the cycle. Some patients have a low plasma progesterone level beyond the 20th day. They presumably had a late ovulatory or anovulatory cycle. The same irregularity may occur in normal subjects in the premenopausal period. Very few ER+ tumors contain PGR at the end of the cycle.

Table 3 shows the average plasma steroid levels and tumor receptor content for estradiol and progesterone. The incidence of ER+ tumors increases from the beginning to the end of the cycle (39 to 76%), while the incidence of PGR+ tumors decreases (34 to 14%). The mean content of ER is lower at the end of the cycle, but the difference is not significant. The variations of the progesterone binding sites are also not significant in this small series of tumors.

The mean plasma progesterone is low during the first decade when the incidence of PGR is the highest. Further, no measurable PGR was observed when plasma progesterone was higher than 100 ng/100 ml (Table 4). The mean progesterone plasma level in patients with ER+ PGR+ tumors was 38 ± 16 ng/100 ml, whereas it was 153 ± 41 in patients whose tumors contained ER only. The difference is significant (\(p < 0.05\)). Plasma estradiol was not different in the corresponding categories of tumors (Tables 4 and 5).

**Postmenopausal Patients.** In normal postmenopausal women, estrogens and progesterone have mainly an adrenal origin (2, 24, 26) and plasma levels are usually low. Estrone is the major circulating estrogen. In the postmenopausal patients the plasma estrogen and progesterone...
Chart 1. A, plasma estradiol and estrogen receptors in relation to the day of the cycle; B, plasma progesterone and PGR's. The different symbols indicate the tumor receptor content.

Table 3

Incidence of ER+ and PGR+ tumors, ER and PGR tumor content, and plasma estradiol and progesterone levels

The menstrual cycle is divided into 3 decades. The incidence of ER+ and PGR+ tumors, the mean ER and PGR tumor content, and the mean plasma estradiol and progesterone levels are calculated for each decade. When the number of cases is small, the range or the individual values are given.

<table>
<thead>
<tr>
<th>Day after last menstrual period</th>
<th>No. of ER+ tumors</th>
<th>Mean ER content (fmol/mg protein)</th>
<th>Mean plasma estradiol (ng/100 ml)</th>
<th>No. of PGR+ tumors</th>
<th>PGR content (fmol/mg protein)</th>
<th>Mean plasma progesterone (ng/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>9/23 (39%)</td>
<td>38 ± 25*</td>
<td>8.9 ± 5</td>
<td>8/23 (34%)</td>
<td>182 ± 77*</td>
<td>50 ± 15* (10-100)</td>
</tr>
<tr>
<td>11-20</td>
<td>9/19 (47%)</td>
<td>36 ± 12</td>
<td>13.5 ± 5</td>
<td>4/19 (21%)</td>
<td>321 ± 269**</td>
<td>267 ± 84** (17-447)</td>
</tr>
<tr>
<td>&gt;21</td>
<td>16/21 (76%)</td>
<td>29 ± 8</td>
<td>8.9 ± 1</td>
<td>3/21 (14%)</td>
<td>179 ± 133**</td>
<td>179 ± 87** (10-800)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>179 ± 87**</td>
<td>179 ± 87** (10-800)</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

NS, not significant.

determinations were recorded in groups corresponding to the different patterns in receptor content, i.e., ER+ PGR+, ER+ PGR-, and ER- PGR- (Table 6). The group ER- PGR+ represents only 2%.

Estrogen values are very similar in the different groups and are characterized by an estrone level higher than the mean observed in normal subjects of the same age (26). In some individual cases the estrone + estradiol value may
reach a level in the same range as those in premenopausal women (Chart 2). Such data reflect the increased adrenal function that we have already described in breast cancer patients (27). It is a common feature in other neoplastic diseases, and it is correlated with the progression of the illness. We observed, however, that the plasma progesterone level is always low (Table 6). It is never higher than the values observed in the follicular phase in premenopausal women.

The mean ER content is $174 \pm 38$ fmoi/mg protein in the tumors from postmenopausal patients, but there is a marked variation depending on whether the tumor contains PGR. The values are $224 \pm 59$ and $123 \pm 42$, respectively, and the difference is statistically significant ($p < 0.05$). This is in agreement with previous observations that ER and PGR tumor contents are positively correlated (18, 25). Moreover, Chart 2 shows for each group (PGR+ and PGR−) that, when the ER content of the tumor is plotted against the corresponding plasma estrogen level, the correlation is significant for the tumors in which PGR is measurable, but it is not significant for the tumors that do not contain PGR.

**DISCUSSION**

In different mammalian species the administration of estradiol stimulates the synthesis of both ER's and PGR's in the endometrium (20, 21), whereas progesterone induces a reverse effect (6, 9, 19, 22). The stimulating role of estrogens on the growth of mammary tumors has long been established. It has recently been demonstrated that they stimulate the synthesis of ER in experimental 7,12-dimethylbenz(a)anthracene-induced tumors. Estrogen action on receptors in vivo is thought to be indirect due to an increase of prolactin secretion (15, 32, 35), but it is believed that PGR is directly estrogen dependent in mammary cancer. This has been demonstrated in experimental tumors (11).

The levels of ER that we have found in tumors from premenopausal patients are similar to those already reported (18, 28). Since the method used measures only unoccupied sites, the low levels that have been observed could be due to the occupancy of receptors by endogenous hormone, which is higher in premenopausal than in postmenopausal women. In fact, bound estradiol may be exchanged and measured in tumors from pre- and postmenopausal patients (21, 29). Such assays have shown that endogenously bound ER exists in both, in agreement with...
the nonnegligible amount of circulating estrone after the menopause. Therefore, the existence of endogenously bound cytosol receptors cannot account for the difference in ER concentration in relation to menopausal status.

In premenopausal patients the tumor estrogen receptor content seems to be lower during the luteal phase, but the difference is not significant. Moreover, it could not be related to the changes of plasma estradiol since it was similar during the first and the third decade in this series of patients.

In normal breast tissue from tumor-bearing breast, the PGR concentration was dependent upon the stage of the menstrual cycle (20) and was lower during the proliferative phase. Nevertheless, it was positive throughout the cycle. According to our data the negative effect of progesterone on its own receptor is more evident since no PGR was detectable in the cytosol when the plasma progesterone was higher than 100 ng/100 ml. Moreover, the highest rate of tumors containing PGR corresponds to the patients with the lowest plasma progesterone levels (Table 5), and the mean highest progesterone levels correspond to the ER+ PGR− and ER− PGR+ tumors. They are significantly different from progesterone levels in the ER+ groups.

Since PGR synthesis is estrogen dependent, it has been suggested that the presence of both ER and PGR might be a selective marker for hormone-dependent tumors (10). Our results suggest that the absence of PGR in tumors from premenopausal patients should be interpreted warily if the plasma level of progesterone is unknown. In these patients the measurement of plasma steroids gave us more precise information on the cycle than the date of the last menstrual period. Such alterations of the cycle are in agreement with other data. First, a low progesterone/estrogen ratio and anovulatory cycles are more frequent in breast cancer patients than in a normal population (17, 33). It has even been suggested that a relatively low progesterone secretion might favor the development of cancer. But breast tumors frequently arise just before the menopause (17), and the existence of irregular cycles is common at this time.

The main difference in receptor content observed between pre- and postmenopausal patients involves ER. After the menopause the mean level of plasma estrone is similar to that of estradiol during the follicular phase. It is conceivable that it is sufficient to stimulate ER directly or indirectly. But this stimulation is not overcome by the effect of progesterone since its plasma level remains low.

In a selected group of postmenopausal patients (ER+ PGR+), there is a significant correlation between plasma estrogen level and the ER content of the tumor. Moreover, the mean ER content is more elevated than in PGR− tumors. That would suggest that estrogens have a role in the stimulation of their own receptors in breast adenocarcinoma, as they do in other target tissues. This is in agreement with the incomplete reactivation of tumor growth by prolactin alone in the absence of estrogen (7). Circulating estrogens of adrenal origin are not negligible in postmenopausal patients, and our data suggest that they are sufficient to stimulate the synthesis of PGR. But PGR may be induced only if estrogen receptors are able to respond to the activation by their own hormone. The presence of PGR, therefore, seems to be a marker of ER integrity. Nevertheless, the lack of PGR in tumors from premenopausal women should be balanced with the corresponding level of circulating progesterone.

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