Role of Pituitary Hormones in the Growth of Human Breast Cancer

Olof H. Pearson,2 Andrea Manni, Mark Chambers, Jerald Brodkey, and James S. Marshall

Departments of Medicine [O. H. P., A. M., M. C., J. S. M.] and Neurosurgery [J. B.], Case Western Reserve University School of Medicine, Cleveland, Ohio 44106

Abstract

Hypophysectomy was performed in 28 women with Stage IV breast cancer who were treated initially with antiestrogens. Six of 13 patients who responded to tamoxifen and 2 of 12 who failed to benefit from tamoxifen obtained remissions from hypophysectomy. The remissions average 11+ months. Three of 8 patients treated initially with antiestrogens have responded to androgen therapy. The results suggest that hormones other than estrogen, which appears to play a major role, may be involved in stimulating the growth of some human breast cancers. Prolactin receptors were detectable in 51% of human breast cancers and were detected in both estrogen receptor-positive and -negative tumors. Preliminary clinical correlations suggest that prolactin receptors will not be useful in predicting response to antiestrogen therapy.

Introduction

Assessment of the hormonal control of breast cancer growth in women has been gleaned from clinical observations involving endocrine manipulations in patients with advanced disease. Tumor regression induced by ovariectomy in premenopausal patients with Stage IV breast cancer led to the concept of estrogen dependence of tumor growth (1, 20). Subsequently, adrenalectomy or hypophysectomy was shown to induce further tumor regression in some women who were previously castrated or were postmenopausal (10, 19, 26). The rationale for adrenalectomy was the concept that the adrenal glands were a second source of hormones and that a further decline in circulating estrogens in castrated or postmenopausal women might induce tumor regression in women with estrogen-dependent cancers. Hypophysectomy was undertaken with the concept that suppression of circulating levels of pituitary hormones such as growth hormone and prolactin might induce tumor regression over and above that obtained from estrogen suppression if the growth of some breast cancers were dependent upon these hormones. In a prospective, randomized study comparing adrenalectomy versus hypophysectomy, Hayward et al. (7) reported a definite superiority of response for hypophysectomy, suggesting that pituitary hormones may also play a role in stimulating tumor growth.

Animal models were developed which have permitted more detailed study of endocrine factors involved in stimulating tumor growth. Huggins et al. (9) developed such a model by feeding to rats a carcinogen, 7,12-dimethylbenz(a)anthracene, which induced breast cancers, most of which were hormone dependent. This animal model has been extensively studied, and in our experience this tumor is primarily prolactin dependent (13, 17, 23). Thus, estrogen was unable to stimulate tumor growth in the absence of the pituitary gland, whereas prolactin was able to stimulate growth of tumors in the apparent absence of estrogens.

In the past few years, antiprolactin and antiestrogen drugs have undergone clinical trials in patients with Stage IV breast cancer in an attempt to develop medical therapy comparable to or perhaps superior to endocrine ablative procedures. Ergot alkaloid drugs have been shown to be effective inhibitors of prolactin secretion in rats (15, 16) and in humans (6). Clinical trials of such drugs in women with metastatic breast cancer yielded only minimal effects on tumor growth despite effective suppression of serum prolactin levels (4, 6, 18). On the other hand, potent nonsteroidal antiestrogen drugs have been developed which bind competitively to the estrogen receptor and thus appear to act by blocking the entry of estrogens into target organs rather than by suppressing secretion. Clinical trials of 2 such compounds have demonstrated that they can induce objective remissions in some patients with breast cancer (2, 3). In our experience with one of these drugs, tamoxifen (I.C.I. Americas, Inc., Wilmington, Del.), we have obtained remissions comparable in incidence and duration to those of surgical hypophysectomy (12). In addition, tamoxifen has induced remissions in some patients who responded previously to hypophysectomy and in whom pituitary hormones were undetectable in the serum but estradiol and estrone were detectable at low levels. These observations suggest that most hormone-responsive breast cancers in women are estrogen dependent and that estrogens are effective directly at the tumor level.

In this report we present observations on the effects of hypophysectomy in patients who were initially treated with antiestrogens. In addition, we have measured prolactin receptors in primary and metastatic breast cancers and have found them to be present in about 50% of these tumors. The results suggest that pituitary hormones may play a role in maintaining the growth of some human breast cancers.

Materials and Methods

Women with Stage IV breast cancer who were treated initially with tamoxifen, 20 mg p.o. every 12 hr, and either failed to benefit or had a remission followed by relapse underwent transsphenoidal hypophysectomy or were treated with halotestin, 10 mg p.o. twice a day. All patients had progressive measurable disease. Remission is defined as objective regression of dominant tumor masses either complete or greater than 50% of the product of 2 diameters
without evidence of progression elsewhere or the development of new lesions for at least 3 months. Recalcification of osteolytic lesions is considered evidence of objective remission. No progression of disease is defined as tumor regression which is less than 50% of 2 diameters with no progression elsewhere for at least 6 months. Failure is defined as greater than 25% increase in the 2 diameters of a tumor after an adequate period of trial (6 weeks).

Primary or metastatic mammary cancers for prolactin receptor assay were obtained from the operating room and promptly frozen in liquid nitrogen. The tissue was kept at −70°C until receptor assays were performed. Prolactin receptors were measured in the following manner.

One g of frozen tumor specimen was pulverized in a Thermovac (Thermovac Industries Corp., Copiague, L. I., N. Y.) and homogenized in a Polytron with two 15-sec bursts at setting 4 in 0.3 M sucrose at 4°C. The homogenate was centrifuged twice at 5000 x g for 15 min at 4°C, and the supernatant was saved for preparation of the cytosol. The pellet was resuspended in buffer (0.25 M sucrose, 0.01 M Tris-HCl, 0.1 M KCl, and 3 mM MgCl2·6H2O, pH 7.4), and protein content was determined by the method of Lowry et al. (11).

Ovine prolactin (NIH-P-S-12) was labeled with 125I by the lactoperoxidase method of Franz and Turkington (5) but separated and recovered via a single 20-hr elution on a 1.5 x 60 cm Sephadex G-100 column in phosphate buffer (10 mM NaH2PO4-Na2HPO4, pH 7.0). The specific activity of labeled prolactin prepared in this manner ranged from 130 to 170 μCi/μg.

About 100,000 cpm of labeled hormone was incubated with 200 μl of particulate fraction (3 mg/ml) with 10 serial dilutions of unlabeled ovine prolactin (0 to 5000 ng/tube) in a final volume of 0.5 ml using buffer (0.025 M Tris-HCl, 10 mM CaCl2, and 0.1% bovine serum albumin, pH 7.6). Incubation was carried out for 16 hr at 23°C. The incubations were terminated by addition of 3 ml of chilled buffer and centrifuged at 200 x g for 30 min, and the supernatant was decanted. The pellets were counted in a gamma counter.

Total binding was the cpm bound in the absence of unlabeled hormone. The cpm in the presence of 500, 1000, and 5000 ng of unlabeled ovine prolactin were averaged and used as the value for nonspecific binding and subtracted from the total binding. Scatchard analysis (21) was performed, and the binding affinity (Kd) was determined. The number of binding sites was determined by extrapolation to the abscissa and expressed as fmol/mg of protein.

The specificity of the receptor assay was tested using a large human breast cancer and conducting parallel incubations with labeled ovine prolactin in the presence of different unlabeled hormones. The results are shown in Chart 1. There was no significant displacement of labeled ovine prolactin by bovine growth hormone, insulin, and LER 907 (follicle-stimulating hormone and luteinizing hormone), whereas significant displacement was obtained with ovine prolactin, human prolactin, and human growth hormone which is known to have lactogenic properties.

The time and temperature at which incubation was carried out were varied with the use of a rat liver particulate fraction in an attempt to determine optimum conditions for the measurement of prolactin binding. The effect of temperature was studied at 4°C, 23°C, and 30°C and time of incubation at 3, 6, and 16 hr. The Kd was quite similar under these varied conditions, but the number of binding sites was found to be maximum for 16 hr at 23°C. There was about a 3-fold increase in the number of binding sites determined after an incubation of 16 hr as compared to 6 hr at 23°C.

Cytosol estrogen receptors in tumor specimens were determined by the method of McGuire and DeLaGarza (14).

Results

Twenty-eight women with Stage IV breast cancer who were initially treated with antiestrogens have subsequently undergone transphenoidal surgical hypophysectomy. The results are shown in Table 1. Of 13 patients who obtained an initial remission from tamoxifen, 6 obtained objective regression, 2 had no progression of disease, and 5 failed to benefit from hypophysectomy. Of 12 patients who failed to benefit from tamoxifen, 2 obtained remissions from hypophysectomy. The remissions in 8 patients have averaged 11+ months.

Eight patients who were initially treated with antiestrogens subsequently received androgen therapy. One of 3 patients who initially responded to tamoxifen later obtained a remission from androgen therapy, whereas 2 of 5 patients in whom antiestrogen therapy failed subsequently benefited from halotestin. The androgen-induced remissions in these 3 patients averaged 11+ months. Of interest is one patient who failed to respond to antiestrogens and subsequently obtained a remission from hypophysectomy lasting 16 months and later responded to androgen therapy lasting 10+ months.

Prolactin receptors were measured in 111 human breast cancers of which 78 were primary and 33 were metastatic lesions. Prolactin receptors were considered to be positive in 51% of the tumors with a mean value of 47 ± 11 (S.D.) fmol/mg protein (range, 10.1 to 347) and a mean Kd of 5.4 ± 0.6 x 10^-10 M (range, 0.6 to 12.7). Specific binding of prolactin (total binding minus nonspecific binding) averaged 3.30 ± 0.5% with a range of 1.01 to 9.22% in 57 tumors which were designated as positive for prolactin receptors.

Of 54 tumors judged to be negative for prolactin receptors...
because of poor Scatchard plots or high dissociation constants, specific binding averaged 1.50 ± 0.14% with a range of 0 to 6.63%. Approximately one-half of these tumors showed more than 1.0% specific binding and might have been considered "positive" (8) if Scatchard plots had not been carried out.

Estrogen receptors were measured in 107 of the above tumors; 39 of 79 estrogen receptor-positive tumors were positive for specific prolactin binding and 16 of 28 estrogen receptor-negative tumors also demonstrated specific prolactin binding. There was no significant correlation between the actual levels of estrogen receptors and the amount of specific prolactin binding.

A preliminary clinical correlation between the presence of estrogen and prolactin receptors in the tumor and response of the patient to antiestrogen therapy has been carried out. The results are shown in Table 2. It is apparent that absence of specific prolactin binding did not preclude a response to antiestrogen therapy in this small series. At present we do not have sufficient data to correlate the response to hypophysectomy after antiestrogen therapy with the presence of specific prolactin binding in the tumor.

Discussion

The excellent remissions induced by antiestrogen in women with Stage IV breast cancer, which appear to be comparable in incidence and duration to those of surgical hypophysectomy, suggest that estrogens play a major role in maintaining the growth of hormone-responsive tumors (12). The fact that antiestrogens may be effective in causing tumor regression after complete hypophysectomy in some patients suggests that estrogens may act directly at the tumor level rather than indirectly via the pituitary gland. The results presented here, which show that hypophysectomy or androgen therapy may induce remissions in some patients after antiestrogen therapy, suggest that other hormonal factors may play a role in stimulating tumor growth. The fact that antiprogesterone drugs produced only minimal antitumor effects in women (18) suggested that prolactin may not be playing a significant role in human breast cancer in contrast to the major role that it has in rat mammary tumors. On the other hand, since human growth hormone is known to have lactogenic properties and since ergot drugs that effectively suppress prolactin do not alter growth hormone secretion, it seems possible that suppression of both growth hormone and prolactin by hypophysectomy might account for the tumor regression observed in these patients. Other possibilities, such as suppression of insulin secretion by hypophysectomy as suggested by Holdaway and Friesen (8), cannot be ruled out as an explanation for the hypophysectomy-induced remissions after antiestrogen treatment.

The mechanism by which pharmacological doses of androgen induce remissions in women with breast cancer is unknown. The fact that they were effective after antiestrogen treatment suggests that the mechanism is not via suppression of estrogen action. Since androgens were effective in one patient after both antiestrogen treatment and hypophysectomy, it seems possible that they may act directly at the tumor level. Androgen receptors have been shown to be present in some human mammary cancers (24, 25). A trial of antiandrogens in women with breast cancer would be of interest.

Our finding of specific prolactin binding in human mammary cancers confirms the observations of Holdaway and Friesen (8). However, these investigators were able to detect only low levels of specific prolactin binding in about 20% of the tumors studied, whereas we found higher levels in more than 50% of breast cancers. We believe that these differences may be related to the methods used to detect prolactin binding. We used a whole particulate fraction as compared to their membrane particulate fraction, we used labeled ovine prolactin instead of human prolactin, and we used a 16-hr incubation time in contrast to 6 hr which we used for our experiments. Table 2 shows the correlation between estrogen and prolactin receptors and response to tamoxifen.

Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Response to tamoxifen</th>
<th>Duration (mos.)</th>
<th>Response to hypophysectomy</th>
<th>Duration (mos.)</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. M.</td>
<td>R&quot;</td>
<td>9</td>
<td>R</td>
<td>28+</td>
<td>Skin, bone</td>
</tr>
<tr>
<td>A. M.</td>
<td>R</td>
<td>16</td>
<td>R</td>
<td>4</td>
<td>Breast, bone</td>
</tr>
<tr>
<td>A. Q.</td>
<td>R</td>
<td>5</td>
<td>R</td>
<td>15+</td>
<td>Breast, skin</td>
</tr>
<tr>
<td>E. B.</td>
<td>R</td>
<td>23</td>
<td>R</td>
<td>10+</td>
<td>Lung</td>
</tr>
<tr>
<td>J. S.</td>
<td>R</td>
<td>29</td>
<td>R</td>
<td>7+</td>
<td>Bone</td>
</tr>
<tr>
<td>L. P.</td>
<td>R</td>
<td>5</td>
<td>R</td>
<td>3+</td>
<td>Lymph node</td>
</tr>
<tr>
<td>D. D.</td>
<td>R</td>
<td>9</td>
<td>NP</td>
<td>10</td>
<td>Bone</td>
</tr>
<tr>
<td>W. K.</td>
<td>R</td>
<td>13</td>
<td>NP</td>
<td>8</td>
<td>Bone</td>
</tr>
<tr>
<td>M. B.</td>
<td>R</td>
<td>4</td>
<td>F</td>
<td>Skin, bone</td>
<td></td>
</tr>
<tr>
<td>S. G.</td>
<td>R</td>
<td>10</td>
<td>F</td>
<td>Skin, bone</td>
<td></td>
</tr>
<tr>
<td>A. Q.</td>
<td>R</td>
<td>12</td>
<td>F</td>
<td>Pleura, bone</td>
<td></td>
</tr>
<tr>
<td>W. W.</td>
<td>R</td>
<td>8</td>
<td>F</td>
<td>Breast, lung</td>
<td></td>
</tr>
<tr>
<td>P. Z.</td>
<td>R</td>
<td>25</td>
<td>F</td>
<td>Bone</td>
<td></td>
</tr>
<tr>
<td>J. F.</td>
<td>NP</td>
<td>11</td>
<td>F</td>
<td>Bone</td>
<td></td>
</tr>
<tr>
<td>E. F.</td>
<td>NP</td>
<td>20</td>
<td>F</td>
<td>Lung</td>
<td></td>
</tr>
<tr>
<td>J. McK.</td>
<td>NE</td>
<td>7</td>
<td>F</td>
<td>Bone marrow</td>
<td></td>
</tr>
<tr>
<td>D. P.</td>
<td>F</td>
<td>R</td>
<td>16</td>
<td>Breast, skin, lymph nodes</td>
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</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>Remission/total no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+ PRL-R+</td>
<td>4/5</td>
</tr>
<tr>
<td>ER+ PRL-R~</td>
<td>4/4</td>
</tr>
<tr>
<td>ER- PRL-R+</td>
<td>0/1</td>
</tr>
</tbody>
</table>

"a R, remission; NP, no progression; NE, nonevaluable; F, failure.

"b ER+, estrogen receptor positive; ER-, estrogen receptor negative; PRL-R+, prolactin receptor positive; PRL-R~, prolactin receptor negative.

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were able to show increased the number of binding sites detectable. We feel that a minimum of 1 g of tumor tissue is necessary for prolactin binding assays, and that multiple point suppression curves with increasing amounts of unlabeled prolactin suitable for Scatchard analysis afford the best opportunity for demonstrating prolactin binding. Results similar to ours have also been reported by Stagner et al. (22) in a preliminary communication.

Clinical correlation between the presence of prolactin receptors in the tumor and response to endocrine therapy is as yet too preliminary to determine whether they will prove to be of value in predicting responsiveness. From the data in Table 2, it seems unlikely that they will be useful in prediction of response to antiestrogens. Whether prolactin receptors may be useful in prediction of response to hypophysectomy after antiestrogen treatment remains to be determined.

References


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