Endocrine Therapy for Advanced Carcinoma of the Breast: Relationship between the Effect of Tamoxifen upon Concentrations of Progesterone Receptor and Subsequent Response to Treatment


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ABSTRACT

In some cell lines and tumors of mammary origin, tamoxifen causes an increase of progesterone receptor (PR) as a result of its partial estrogen agonist activity. In this study we have assessed the effect of tamoxifen on PR in patients with advanced carcinoma of the breast in order to test if those with a rise in PR are more likely to respond to endocrine therapy. PR was measured before and a median of 13 days after treatment with tamoxifen in a group of 52 patients with either locally advanced (n = 28) or recurrent (n = 24) carcinoma of the breast. Controls were a group of patients with operable disease who had two biopsies with no intervening tamoxifen (n = 51) or with intervening tamoxifen (n = 58).

In the test group PR was higher in the second biopsy than the first in 21 patients, and 19 of these responded to continued endocrine therapy (90%). In the remaining 31 patients PR was either lower in the second biopsy (n = 19) or was negative in both biopsies (n = 12), and 11 of the total of 31 patients (35%) responded to continued endocrine therapy. The prediction of response and time to progression was better when both biopsies were taken into account than either the first or the second alone. The prediction of survival was similar for the group selected by an increase in the second biopsy and the group with PR present in the second biopsy.

The controls without tamoxifen showed a marked variation in the level of PR in the first and second biopsies, suggesting heterogeneity of PR across the tumors studied. However, the PR level was significantly higher in the second biopsy in the controls given tamoxifen and in the test group compared with those with no intervening treatment (p = 0.031).

This study indicates that some effect of tamoxifen upon PR can be demonstrated in human mammary tumors in vivo and that, by taking a second biopsy for PR estimation during treatment with tamoxifen, a more precise indication of subsequent response is obtained. The value of a single estimation of PR before treatment on secondary deposits is limited, and if one biopsy only is performed, it is of greater predictive value if taken after a few days treatment with tamoxifen.

INTRODUCTION

Approximately one-half of ER$^+$-positive human mammary tumors respond to endocrine therapy. In ER-positive, endocrine-unresponsive tumors the receptor is thought to be defective in some way and therefore not capable of initiating an effect in response to either estrogen or antiestrogens. A product of estrogen action, if present, might indicate an intact receptor mechanism and a responsive tumor, and several candidate markers have been suggested, including estrogen-regulated mRNAs (1–3) and intracellular and secreted proteins (4–7). Horwitz and McGue (8) suggested that PR might be a suitable marker, and this led to the widespread introduction of assays for this protein. As shown in the previous paper and described in a review by McGuire and Clarke (9) PR does improve the prediction of response to endocrine therapy, but approximately one-quarter of tumors behave inappropriately.

Two possible reasons for this are that, in PR-negative, but -responsive tumors, there is insufficient endogenous estradiol to stimulate PR synthesis, and in PR-positive, -unresponsive tumors, PR may be synthesized independently and not be indicative of functional ER.

Unlike several other estrogen-induced products, PR synthesis is stimulated by the antiestrogen tamoxifen in human mammary tumor cells. In the MCF 7 cell line, tamoxifen at low concentrations (<0.1 μM) acts as a potent estrogen and induces PR over 24 to 48 h, the levels of which increase 4- to 10-fold over 4 to 6 days (10). As tamoxifen is commonly used for the first endocrine treatment in advanced cancer of the breast it can also be used to test if PR synthesis is also stimulated in vitro. In two early reports biopsies of skin metastases were taken before and a few days after the administration of tamoxifen, and in some tumors the second PR value was greater than the first (11, 12). In this study we have measured PR before and during treatment with tamoxifen and related the change in PR to the subsequent response to continued endocrine therapy with the aim of improving the prediction of response by reducing false negative and false positive PR estimations.

PATIENTS AND METHODS

Patients. A total of 161 patients, in 3 groups, had 2 biopsies of their tumor for measurement of PR. Group A, the test group, consisted of 52 patients with locally advanced primary tumors or recurrent chest wall disease. They were treated with tamoxifen in the interval between the two biopsies, endocrine therapy was continued afterwards, and response was assessed according to criteria of the International Union against Cancer (13). Group B, untreated controls, had 51 patients with operable primary tumors who were given no treatment in the interval between biopsies. Group C, treated controls, consisted of 58 patients with operable primary tumors treated with tamoxifen in the interval between biopsies. The median time between biopsies was 13 days (range, 5 to 31 days) for Group A and 8 days (range, 5 to 14 days) for Groups B and C.

Tamoxifen. Groups A and C were treated with tamoxifen in the interval between biopsies. Thirty-one patients in Group A and all patients in Group C were given tamoxifen (40 mg) four times per day, as a loading dose on the day after the first biopsy, and then subsequently given 20 mg per day until the time of the second biopsy. Twenty-one patients in Group A were not given a loading dose and had 20 mg on each day between biopsies. In Group A, with the exception of 2 patients who were continued on medroxyprogesterone acetate, tamoxifen treatment was continued until progression of disease. All but 8 patients were previously untreated for advanced disease: 4 had had ovarian ablation; 3 were treated with tamoxifen; and 1 had chemotherapy. There was an interval of at least 6 wk between these treatments and the start of this study.

Biospy Techniques. For Group A patients, biopsies were performed either with a Tru-cut needle (Travenol) or by surgical excision of a...
small fragment of tumor. All the first biopsies for patients of Groups B and C were performed with a Tu-cut needle: small pieces of tissue were removed from the primary tumor at mastectomy or wide local excision for the "second biopsy" sample. All tissues were placed immediately in, and stored in, liquid nitrogen until the time of assay.

Receptor Assay. With the exception of three tumors in Group A where ER and PR were measured using the dextran-coated charcoal technique (14), all assays were performed by isoelectric focusing as previously described (15, 16). For both ER and PR the sample was regarded as positive if 5 or more fmol/mg of cytosol protein were measured using the DCC assays and any positive value of appropriate pl using isoelectric focusing.

Evaluation of Response. Response was assessed according to criteria of the International-Union against Cancer (13) with the exception that stable disease had to be present for more than 6 mo for patients to qualify for this category.

Statistical Methods. The log rank test (17) was used to compare survival and time to progression data. The Mann-Whitney U test and Wilcoxon’s matched pairs signed rank test were used, where appropriate, on the PR values (18).

RESULTS

Patients. The characteristics of the group of 52 patients with advanced disease are outlined in Tables 1 and 2. Twenty-eight patients had locally advanced disease and 24 had recurrent disease as a prerequisite for entering this study. Sixty-two % of patients had additional sites of disease in bone and lung, but there was none with liver metastases.

The proportion of ER-positive tumors (67%) was similar to the proportion for all patients in our data base, but the proportion of PR-positive tumors (71%) is higher, reflecting a selected population with local disease. The response rate (complete plus partial responses) was low (21%) but similar for the locally advanced (22%) and recurrent groups (20%). When stable disease was included the overall response in those with locally advanced disease was 67% and with recurrent disease, 48%.

When the first biopsy was considered the prediction of response according to receptor content was better in locally advanced tumors (80% of PR-positive tumors responded) compared with recurrent tumors (41% of PR-positive tumors responded). A high proportion of ER-negative (6 of 17, 35%) and PR-negative (6 of 15, 40%) tumors responded (Table 2).

Effect of Tamoxifen. Tamoxifen was administered immediately after the first biopsy and was continued until there was progression of disease (with the exception of two patients continued on MPA). The second biopsy was performed at a median of 13 days (range, 5 to 31 days) after the first. PR was higher in the second biopsy compared with the first in 21 tumors (40%), lower in the second biopsy in 19 tumors (37%), and negative in both biopsies in 12 tumors (23%) (Fig. 1).

Nineteen of the 21 (91%) tumors where PR was higher in the second biopsy subsequently responded to continued tamoxifen or MPA (Table 3). Three tumors were PR negative in the first biopsy and PR positive in the second, and all three responded. The two tumors (one locally advanced, one recurrent) in this group which failed to respond progressed rapidly, and the patients died after 11 and 10 mo, respectively. Eight of the 19 (42%) tumors where PR was lower in the second biopsy responded to continued tamoxifen: in 9 tumors the second biopsy was PR negative; and 3 (33%) of these responded. There was no significant difference in ER level between the tumors with higher or lower ER (mean, 69 and 82 fmol/mg, respectively). Three of the 12 (33%) tumors where both biopsies were negative responded. When the three groups were considered (i.e. higher PR in the second biopsy, lower, or double negative), there were similar proportions of patients with advanced disease in each group, similar proportions given loading dose tamoxifen, and similar median times to the second biopsy (median, 13, 13, and 15 days, respectively; Fig. 1).

When the PR value in the second biopsy was considered alone, 24 of 31 (74%) PR-positive tumors responded, and 6 of 21 (29%) PR-negative tumors responded. This discrimination was better than when the first biopsy alone was considered, but not when both biopsies were considered (Table 3). The predictive value of double receptor phenotypes or of receptor levels was inferior to the change in PR (Table 4).

Time to Progression and Survival. There was no significant difference between the time to progression for PR-positive and PR-negative tumors when the first biopsy was considered (Fig. 2A). PR-positive tumors had a significantly longer time to progression than PR-negative tumors when the second biopsy alone was considered (Fig. 2B; P < 0.002). Tumors where the PR value was higher in the second biopsy than the first had a longer time to progression than either those where the second PR value was lower or where both were negative (Fig. 2C; P <
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Table 3 Response according to PR in the first biopsy, PR in the second biopsy, and change of PR between the two biopsies

<table>
<thead>
<tr>
<th>Response</th>
<th>Complete response</th>
<th>Partial response</th>
<th>Stable disease</th>
<th>Progressive disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>First biopsy PR+ 24/37 (62)</td>
<td>3 8 7 19</td>
<td>14 38 13 35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR- 6/15 (40)</td>
<td>1 7 5 33</td>
<td>9 60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second biopsy PR+ 24/31 (74)</td>
<td>3 10 8 26</td>
<td>13 42 7 22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR- 6/21 (29)</td>
<td>6 29 15 71</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two biopsies PR+ 19/21 (91)</td>
<td>2 9 6 29</td>
<td>11 52 2 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR- 8/19 (42)</td>
<td>1 5 2 11</td>
<td>5 26 11 58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR- in both 3/12 (25)</td>
<td>3 25 9 75</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* PR+, PR positive; PR-, PR negative.
* Numbers in parentheses, percentage.

Fig. 1. PR values in the first (1) and second (2) biopsy. ○, responsive tumor; ●, unresponsive tumor. Loading dose tamoxifen. Numbers refer to the time in days between the biopsies.

There was no significant difference between the latter two groups.

There was no significant difference in survival when PR-positive and PR-negative tumors in the first biopsy were considered (Fig. 3A), but there was when the second biopsy alone was considered (Fig. 3B; P < 0.01). The survival of patients showing tumors with a rise or a fall of PR was similar and significantly different from those where both biopsies were PR negative (Fig. 3C; P < 0.005).

Controls. The proportion of patients with a higher value of PR in the second biopsy was the same in the untreated controls (21 of 51, 41%) compared with the two groups treated with tamoxifen (treated controls, 27 of 58, 46%; test group, 21 of 52, 40%; Table 5). However, in the tumors where the second PR value was greater than the first, those treated with tamoxifen had higher mean PR values than untreated controls (Table 6).

The mean for the test group (Group A) was 237 fmol/mg of cytosol protein and for the tamoxifen-treated controls (Group B) was 318 fmol/mg of cytosol protein compared with 71 fmol/mg of cytosol protein for the untreated control group (Group C). P for Group A versus Group B = 0.029, and for Group B versus Group C = 0.031. The proportion of second biopsies with PR values > 40 fmol/mg of cytosol protein was 16 of 21 (76%) for Group A, 9 of 21 (43%) for Group B, and 21 of 27 (78%) for Group C.
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Fig. 3. Survival according to PR measurement on the first biopsy (A), the second biopsy (B), and when both biopsies are considered (C). —, PR negative; +, PR positive; †, PR > in second biopsy; ‡, PR > in first biopsy.

Table 5 Proportions of patients where the second PR value was higher than the first or lower than the first or where both values were negative in the test group, the untreated controls, and the tamoxifen-treated controls

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>%</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>52</td>
<td>51</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>PR higher</td>
<td>21</td>
<td>40</td>
<td>27</td>
<td>46</td>
</tr>
<tr>
<td>PR lower</td>
<td>19</td>
<td>37</td>
<td>27</td>
<td>17</td>
</tr>
<tr>
<td>PR negative in both</td>
<td>12</td>
<td>23</td>
<td>31</td>
<td>21</td>
</tr>
</tbody>
</table>

Table 6 Mean and median values for PR in the second biopsy where this value was higher than in the first biopsy in the test group, the untreated controls, and the tamoxifen-treated controls

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>%</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>21</td>
<td>21</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>237 ± 78</td>
<td>72 ± 21</td>
<td>318 ± 135</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>103</td>
<td>33</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>7–1285</td>
<td>4–300</td>
<td>14–2947</td>
<td></td>
</tr>
<tr>
<td>&gt;40 fmol</td>
<td>16</td>
<td>9</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

* P values: Group A versus Group B, 0.029; Group A versus Group C, not significant; Group B versus Group C, 0.031.

DISCUSSION

In this study we have shown that there is a group of tumors where PR is higher in biopsies taken during tamoxifen treatment when compared with a pretreatment biopsy, and that these tumors have a high probability (91%) of responding to continued endocrine treatment. Namer et al. (11) studied 25 patients with cutaneous metatases and measured PR before and after 1-week treatment with tamoxifen; in 12 (48%) the second PR value was higher than the first; in 4 (16%) the second was lower; and in 7 (28%) both values were negative. No data concerning the subsequent response to treatment were given, but it is of interest that the proportions of tumors in each group are similar to our own. Only one of their tumors where PR was higher in the second biopsy was ER negative (1 of 12, 8%), whereas 4 (4 of 21, 19%) of ours were, indicating that the effect is not confined to ER-positive tumors. Waseda et al. (12) studied 20 patients: 11 were ER positive; and 7 of these PR positive. After 1- to 2-wk treatment with tamoxifen there was a higher PR in the second biopsy in 3 of 4 tumors, whereas in 3 tumors treated for longer than 2 wk the second PR value was lower than the first. The timing of the second biopsy did not appear to be critical within the limits of our study.

The median and range of times to the second biopsy were the same in those with a higher or lower value in the second biopsy or if both were negative; the longest time to the second biopsy was 35 days, and the PR value in the first biopsy was 33 fmol/mg of cytosol protein and in the second was 121 fmol/mg of cytosol protein. In a single case report Degenshein et al. (19) treated a 55-yr-old woman with an ER-positive, PR-negative tumor with stilbestrol (15 mg/day) for 3 days and rebiopsied the same lesion, and in this case the PR value was 1000 fmol. No data were given concerning the subsequent response of this patient, and as far as we are aware, the study reported here is the first to relate a change in PR level to the subsequent response to endocrine therapy in human cancer of the breast.

In our study a higher PR value in the second biopsy was associated with a high response rate and a consequently significantly longer time free from progression of disease compared with the group with a lower PR value in the second biopsy or where both were negative. When the first PR value was considered alone there was no predictive value for response or time to progression, whereas the second PR value alone predicted response and time to progression nearly as well as when both receptors were considered; this is because three responders, who were originally PR negative, converted to PR positive after tamoxifen, and 6 nonresponders changed from PR positive in the first biopsy to PR negative in the second. The change from PR negative to PR positive may reflect a response to tamoxifen stimulation, whereas a change from PR positive to PR negative, while on tamoxifen, presumably reflects a tumor with marked heterogeneity for PR with positive and negative areas coexisting and consequently unresponsive to tamoxifen.

The first biopsy did not discriminate for survival, but again PR-positive second biopsies had a similar survival pattern to both patients with an increase and a decrease of PR when two biopsies were considered. It is not clear why those with a lower PR in the second biopsy survived well. It may be that this group have very slowly progressive but largely unresponsive tumors. Although this study suggests that a second PR value higher than the first is of predictive value for subsequent response to endocrine therapy, the results should be interpreted with caution. The control group not treated with tamoxifen had a similar proportion of patients with a higher second PR value due to heterogeneity of PR across tumors as shown in the previous paper. However, because it was possible to demonstrate that the mean PR value was higher in the second biopsies of both the groups treated with tamoxifen, it suggests that tamoxifen is having some effect upon PR synthesis. The most appropriate controls would have been tamoxifen-untreated patients with advanced disease, but we did not feel that it was ethically justifiable to delay definitive treatment in this group of patients.

Despite two measurements for PR several patients had values which were inappropriate to the subsequent response. Two patients in the group where the second PR value was higher than the first had rapidly progressive disease while on tamoxifen; one failed to respond to second endocrine therapy with aminoglutethimide and died after 11 mo with progressive disease on the chest wall and in bone, and the other failed to respond to two combination chemotherapy regimens and died.
after 10 mo. PR was 15 and 17 fmol in the first biopsy and 102 and 244 fmol in the second biopsy in the two patients, respectively. It is possible that tamoxifen either had no effect upon, or stimulated the growth of, these tumors but was still capable of stimulating the synthesis of PR. Indirect evidence from in vitro experiments suggests that this may occur. In an MCF-7 clonal cell line, R₃⁷ (20, 21) made resistant to tamoxifen by continued subculture in the presence of tamoxifen, the drug no longer inhibited growth but remained capable of stimulating PR synthesis. In a separate study the growth of tumor cells taken from patients resistant to tamoxifen was shown to be stimulated by tamoxifen in vitro (22). In both of these studies tamoxifen resistance occurred in the presence of tamoxifen, but it is possible that this could arise de novo and be responsible for the failure to respond in the two patients outlined above. Three patients of the 12 where both PR values were negative responded to therapy, and this suggests that, although PR can indicate responsiveness, it is not essential for response.

This study indicates that some effect of tamoxifen can be demonstrated upon human mammary tumors in vivo and that, by taking a second biopsy for PR estimation after 1- to 2-wk treatment with tamoxifen, a more precise indication of subsequent response is obtained. The value of a single estimation of PR before treatment on secondary deposits is limited, and if one biopsy, only, is performed, it is of greater predictive value if taken after a few days of treatment with tamoxifen. Clearly the results obtained apply only to patients with biopsiable and evaluable disease. We cannot determine whether similar changes occur in PR values in response to tamoxifen at other sites because of the difficulties of biopsy.

REFERENCES


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