Effects of Tamoxifen Adjuvant Therapy and a Low-Fat Diet on Serum Binding Proteins and Estradiol Bioavailability in Postmenopausal Breast Cancer Patients

David P. Rose, Rowan T. Chlebowski, Jeanne M. Connolly, Lovell A. Jones, and Ernst L. Wynder

American Health Foundation, Valhalla, New York 10595 [D. P. R., J. M. C., E. L. W.]; Harbor-UCLA Medical Center, Torrance, California 90509 [R. T. C.]; and University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030 [L. A. J.]

ABSTRACT

Serum was collected at intervals from postmenopausal breast cancer patients to determine the effects of tamoxifen adjuvant therapy and a low-fat dietary intervention, alone and in combination, on sex hormone-binding globulin (SHBG) concentrations and circulating estradiol bioavailability. Serum corticosteroid-binding globulin and follicle-stimulating hormone were also assayed as indicators of patient compliance to tamoxifen therapy. The immunoreactive SHBG concentration was higher (P < 0.001) in 22 patients who had been treated with tamoxifen for 6-36 weeks when first sampled, compared with 27 who were not receiving tamoxifen therapy. Tamoxifen also produced a reduction in the percentage non-protein-bound estradiol (P < 0.001) and percentage albumin-bound estradiol (P < 0.01), the two biologically available fractions, and a corresponding increase in the percentage SHBG-bound estradiol (P < 0.01). A longitudinal study of 7 patients showed significant reductions in the percentage of albumin-bound estradiol and an increased percentage of SHBG-bound estradiol, after 3-6 months of tamoxifen; after 12-18 months there was also a significant decrease in the non-protein-bound estradiol fraction. We conclude that in postmenopausal breast cancer patients the redistribution of circulating estradiol, with reduced bioavailability, provides an additional mechanism to those demonstrated previously for the therapeutic activity of tamoxifen.

Another 12 patients receiving tamoxifen and 8 who were not were followed for 6-12 months on a low-fat diet (fat comprised 20% of the total calories). The dietary intervention had no effect on the serum SHBG concentration or the estradiol distribution.

Although tamoxifen increased the serum corticosteroid-binding globulin and partially suppressed the follicle-stimulating hormone concentrations, the responses obtained were less consistent compared with those of the SHBG levels.

INTRODUCTION

Combination chemohormonal adjuvant therapy which includes the antiestrogen tamoxifen delays recurrence and increases survival in premenopausal breast cancer patients, while tamoxifen alone has been shown to be effective in those beyond the menopause (1-4). Although its therapeutic efficacy is due to its activity as an antiestrogen, tamoxifen also possesses some estrogen-like properties. For example, it exerts an estrogen-like stimulatory effect on the vaginal epithelium (5), suppresses serum gonadotropin levels in postmenopausal women (6), and, like estrogens, induces the hepatic synthesis of several proteins, including SHBG3 (7-10) and CBG (7).

In premenopausal patients, tamoxifen causes an increase in circulating estrogen levels (6, 8, 11-13), and it has been suggested that this may have an adverse effect on the therapeutic efficacy of the drug (13), even though the tamoxifen-induced elevation in SHBG may result in the enhanced binding of estradiol (8). After the menopause, tamoxifen does not cause an elevation in the plasma estrogens (6, 14), and so there is a greater likelihood that by increasing the serum SHBG binding capacity tamoxifen reduces the level of biologically available estrogen. Such an effect might provide an additional mechanism for the antiestrogenic therapeutic activity of the drug.

A low-fat dietary intervention has also been proposed as an adjuvant therapy in postmenopausal breast cancer (15), the rationale for which is based in part on the reduction in serum estrogen concentrations which occurs when postmenopausal women are placed on a low-fat diet (16-18). Moreover, it was reported that, in men, transfer to a low-fat diet causes an increase in the plasma SHBG and a reduction in the free testosterone fraction (19).

In the present report, we describe the effects of tamoxifen adjuvant therapy and a low-fat dietary intervention, alone and in combination, on serum immunoreactive SHBG concentrations and estrogen bioavailability in postmenopausal breast cancer patients. Serum CBG and FSH were also assayed as potential indicators of patient compliance with antiestrogen therapy.

MATERIALS AND METHODS

The patients were all participants in the feasibility phase of the multicenter WINS who had been recruited from either Harbor-UCLA Medical Center (Los Angeles, CA; R. T. Chlebowski) or through the clinical collaborators at the American Health Foundation (Valhalla, NY; E. L. Wynder). Informed consent was obtained from all participants. They were all over 50 years of age and postmenopausal, having had no menstrual periods for a minimum of 12 months, with Stage I to IIIa localized, resected, breast cancer. Entry into the study was required to occur within 180 days of diagnosis, at which time they were randomized to either a dietary intervention group, the goal for which was to reduce fat consumption to 15-20% of total calories, or to the dietary control group. Systemic adjuvant chemotherapy and/or tamoxifen therapy was prescribed according to the current practice of the responsible physician. The dietary aspects of the trial and issues of compliance with the low-fat diet have been discussed elsewhere (15, 20, 21).

Blood samples for serum assays were collected at the time of randomization and at intervals thereafter for a maximum of 18 months. The sera were shipped to the Endocrine Laboratories packed in dry ice and were stored at −70°C until assayed. The maximum delay between sample collection and the assays was 18 months. There were 27 patients who were not treated with tamoxifen; 22 had been receiving the antiestrogen for at least 6 weeks at the time of their entry into the low-fat trial, and another 7 commenced tamoxifen adjuvant therapy after the first blood sample had been obtained.

Assay Methods. Serum SHBG and CBG were determined by radioimmunoassays using reagents produced by Techland (Liege, Belgium) and purchased from Wein Laboratories, Inc. (Sucasunn, NJ). The SHBG assay had a sensitivity of 6.00 nmol/liter and an interassay coefficient of variation of 9.6%. The corresponding values for the CBG assay were 2 µg/ml and 5%. The CBG assays were performed on 21 tamoxifen-treated patients and 22 who were not receiving antiestrogen
therapy. Serum FSH was also measured by radioimmunoassay, using kits purchased from ICN Biomedicals, Inc. (Costa Mesa, CA).

The percentage NPBE and percentage ABE were determined using a previously described isodialysis procedure (22). Briefly, 450-μl serum samples were incubated with \(^{14}C\)glucose and \(^{3}H\)estradiol for 1 h at 37°C. Duplicate 200-μl aliquots were pipetted into modified 12 x 75 mm baked test tubes, closed at one end with a specially treated dialysis membrane and open at the other end, which were then placed in baked minivials. These minivials contained 3 Whatman no. 1 filter disks. They were capped and centrifuged at 37°C for 1 h at 3000 rpm in a Beckman JGB low-speed centrifuge, after which the inner tubes were removed and 30 μl of serum were taken and placed in a separate minivial. Double-distilled water (350 μl) was added to each minivial, and this was then vortexed. After 15 min, scintillation fluid was added, and the contents of the vials was counted in a refrigerated Beckman LS7500 scintillation counter. The percentage NPBE was calculated from \(3H:14C\) (outside)/\(3H:14C\) (inside).

The percentage ABE was determined by comparing the percentage NPBE assayed in untreated and heat-treated serum (23). When serum is heated at 60°C for 1 h at least 90% of the SHBG binding, but not the albumin binding, is destroyed. Thus, the percentage of estradiol that interacts with the heat-stable albumin in the absence of high-affinity binding to SHBG can be estimated by determining the percentage NPBE in heat-treated serum samples. The percentage SHBG-E was calculated by the difference

\[
100 - (\% \text{ ABE} + \% \text{ NPBE}).
\]

The mean intraassay coefficient of variation for the percentage NPBE was 10.0%, and a comparison of the percentage NPBE and percentage ABE assays of control sera stored for more than 1 year showed coefficients of variation of less than 12 and 8%, respectively.

Statistical Evaluation. The nonparametric Mann-Whitney \(U\) test was used for comparisons between the treatment groups. For comparisons of paired data, Student’s paired \(t\) test was used after logarithmic transformations had been performed to reduce the deviations from a normal distribution.

RESULTS

Serum SHBG. The serum SHBG concentrations were significantly higher in the group of 22 patients who had been receiving tamoxifen for periods ranging from 6 to 36 weeks compared with those in 27 untreated patients (Table 1). Eight of the tamoxifen-treated patients assigned to the control dietary group [treatment duration at entry, 14 ± 2 (SD) weeks] and 9 assigned to the low-fat intervention (treatment duration at entry, 12 ± 4 weeks) were followed for 12 months, over which time both groups showed further elevations in their serum SHBG concentrations (Fig. 1A). There were additional 7 patients whose adjuvant tamoxifen therapy commenced after collection of the preintervention blood sample. Their serum SHBG levels were increased significantly (\(P < 0.01\)) after 3–6 months of tamoxifen therapy, but in this group no further elevation in the mean SHBG concentration was evident after 12–18 months of treatment (Table 2).

Twenty patients who did not receive antiestrogen therapy were followed over a 12-month period; 10 had been randomized to the nonintervention dietary group, and 10 were consuming a low-fat diet (fat comprised 20% of the total calories). There was no demonstrable effect of reduced dietary fat intake on the serum SHBG concentrations (Fig. 1B).

Serum CBG and FSH. The serum CBG concentrations were significantly higher (\(P < 0.001\)) in the 21 tamoxifen-treated patients studied compared with the 27 patients who were not receiving antiestrogen therapy (Table 1).

Serum FSH levels were reduced significantly (\(P < 0.01\)) in the 22 tamoxifen-treated patients compared with the 26 patients assayed who had not received tamoxifen adjuvant therapy at entry into the low-fat dietary intervention study (Table 1). However, despite the overall trend for tamoxifen administration to be associated with elevations in serum SHBG and reductions in serum FSH, there was no correlation between these for the entire tamoxifen-treated group (\(r = 0.001\)).

Serum Estradiol Distribution. The percentage NPBE, ABE, and SHBG-E in sera from the 22 tamoxifen-treated and 27 untreated patients assayed before dietary intervention are summarized in Table 3. Tamoxifen administration produced significant reductions in percentage NPBE (\(P < 0.001\)), percentage ABE (\(P < 0.01\)), and the biologically available fractions and a corresponding increase in the percentage SHBG-E (\(P < 0.01\)).

There were strong inverse correlations between the percentage NPBE and the percentage SHBG-E (Fig. 2: \(r = -0.500; \ P < 0.025\) and percentage NPBE and immunoreactive SHBG concentrations (Fig. 3: \(r = -0.588; \ P < 0.005\)). In the group

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**Table 1** Serum SHBG, CBG, and FSH levels (mean ± SD) in tamoxifen-treated and untreated patients before entry into the low-fat dietary intervention trial

<table>
<thead>
<tr>
<th>Assay</th>
<th>No tamoxifen</th>
<th>Tamoxifen-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum SHBG (nmol/liter)</td>
<td>52.2 ± 26.9 (27)*</td>
<td>100.4 ± 35.4* (22)</td>
</tr>
<tr>
<td>CBG (μg/ml)</td>
<td>45.7 ± 6.0  (22)</td>
<td>61.2 ± 8.8* (21)</td>
</tr>
<tr>
<td>FSH (mU/ml)</td>
<td>95 ± 51      (26)</td>
<td>61 ± 21* (22)</td>
</tr>
</tbody>
</table>

* The number of patients in each group is given in parentheses.
* Significantly different from the group not receiving tamoxifen (Mann-Whitney test): \(P < 0.001\).
\(c \ P < 0.001\).
with tamoxifen for 3–6 months resulted in significant reductions in percentage ABE ($P < 0.05$), with corresponding increases in the percentage SHBG-E ($P < 0.05$); the trend toward a reduction in the percentage NPBE did not achieve statistical significance. These alterations in estradiol distribution were even more pronounced after 12–18 months of therapy, with a significant decrease in the NPBE fraction ($P < 0.01$), despite no further change in the mean immunoreactive serum SHBG concentration (Table 2).

Another 12 patients who had already been receiving tamoxifen therapy for 1–4 months and 8 who were not were studied prior to dietary intervention and again after 6–12 months on a low-fat diet. The reduced dietary fat intake alone had no effect on the estradiol distribution, while the changes with prolonged tamoxifen administration did not appear to be modified by low-fat consumption (Table 4).

**DISCUSSION**

The present study confirms previous reports that tamoxifen therapy causes an elevation in circulating SHBG concentrations (7–10), presumably because of a direct estrogen-like effect on the hepatic synthesis of the binding protein. Rose and Davies (8) studied 10 premenopausal breast cancer patients whose menstrual cycles were maintained during adjuvant chemotherapy and in whom the tamoxifen component had produced an elevation in the total plasma estrogen levels. While the increase in plasma SHBG associated with the tamoxifen resulted in a significant reduction in the percentage NPBE, the relative increases in the total plasma estrogens exceeded the SHBG estradiol binding capacity, and so there appeared to be a persistent elevation in the circulating biologically available estrogens. This interpretation was supported by an earlier report that the administration of tamoxifen to premenopausal women produces an increase in the urinary estrogen excretion (11), which would not occur if the elevated plasma estrogens were all SHBG-bound.

Jordan et al. (10) reported an increase in plasma SHBG in premenopausal breast cancer patients who had received adjuvant combination chemotherapy plus tamoxifen followed by long-term tamoxifen alone, compared with a similar group of patients treated with combination chemotherapy alone. In a second study by the same group of investigators (24), 8 premenopausal patients who were receiving long-term adjuvant therapy with tamoxifen alone showed no difference in plasma SHBG concentrations compared with 12 healthy premenopausal women, but significant elevations in plasma estrogens. While the reason for this discrepancy is not evident, it implies, again, that tamoxifen can produce an increase in biologically available circulating estrogens in premenopausal women.

The situation is different in postmenopausal women, because tamoxifen does not cause an increase in the total serum estrogens (6, 14). Jordan et al. (10) pointed out that although the

<table>
<thead>
<tr>
<th>Assay</th>
<th>No tamoxifen (n = 27)</th>
<th>Tamoxifen-treated (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage NPBE</td>
<td>1.599 ± 0.372</td>
<td>1.264 ± 0.237*</td>
</tr>
<tr>
<td>Percentage ABE</td>
<td>52.36 ± 11.34</td>
<td>43.06 ± 7.22*</td>
</tr>
<tr>
<td>Percentage SHBG-E</td>
<td>46.02 ± 11.63</td>
<td>55.67 ± 7.33*</td>
</tr>
</tbody>
</table>

* Significantly different from the group not receiving tamoxifen (Mann-Whitney test; $P < 0.001$).

**Table 4** Percentage estradiol distribution (mean ± SD) in patients before and after low-fat dietary intervention, with or without tamoxifen therapy

<table>
<thead>
<tr>
<th>Assay</th>
<th>No tamoxifen therapy (n = 8)</th>
<th>Tamoxifen therapy (n = 12)</th>
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<tbody>
<tr>
<td></td>
<td>Pre-diet</td>
<td>Diet 6–12 months</td>
</tr>
<tr>
<td>Percentage NPBE</td>
<td>1.489 ± 0.209</td>
<td>1.639 ± 0.323</td>
</tr>
<tr>
<td>Percentage ABE</td>
<td>48.20 ± 8.32</td>
<td>50.16 ± 12.05</td>
</tr>
<tr>
<td>Percentage SHBG-E</td>
<td>50.32 ± 8.50</td>
<td>48.20 ± 12.32</td>
</tr>
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† For statistical analysis by Student's paired $t$ test the results were transformed to log$_{10}$ values.

‡ These patients had been receiving tamoxifen therapy for 1 to 4 months when they commenced the low-fat dietary intervention.

§ Significantly different from the corresponding pre-dietary intervention value: $P < 0.05$. 

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circulating levels of estradiol and estrone are low after menopause, they are still considered to provide the support essential for the growth of hormone-dependent breast cancers. In this situation, they suggested, tamoxifen might effectively reduce the biologically available plasma estrogens by inducing SHBG synthesis and consequently enhancing estrogen-binding capacity.

By showing that tamoxifen therapy produces significant reductions in percentage NPBE and percentage ABE, the two fractions of the serum estrogens which are considered to be biologically available to target cells (25), our present results support the concept proposed by Jordan et al. (10). In addition, we also observed a corresponding elevation in the percentage SHBG-E and negative correlations between the percentage NPBE and both the SHBG-E and the immunoreactive serum SHBG concentrations in the tamoxifen-treated patients.

A number of case-control studies have shown that the percentage NPBE and percentage ABE are elevated in breast cancer patients (26-31), and such increases in bioavailable estrogens have also been associated with recognized or suspected epidemiological risk factors for the disease (31-34). Among these, obesity is a risk factor for postmenopausal breast cancer (35) and results in reduced serum SHBG and percentage NPBE and percentage SHBG-E levels and elevations in the percentage NPBE and percentage ABE in healthy women, although an increase in the biologically available serum estradiol fractions is evident in breast cancer patients even when these are compared with controls matched for body weight (26, 29).

Tamoxifen is being evaluated for the chemoprevention of breast cancer (36-38), and in postmenopausal women the redistribution of circulating estrogen among the plasma fractions provides one of several potential mechanisms for its efficacy. However, given the elevations in the serum estrogens which occur when tamoxifen is given prior to menopause and the indication that a proportion of this is biologically available (8), it is much less certain that such an approach to prevention is appropriate for premenopausal women.

Reed et al. (19) reported that when 6 normal men were transferred from a diet providing 100 g of fat/day to an isocaloric one containing <20 g of fat/day for 2 weeks, there was a significant elevation in the plasma SHBG concentration, with an associated reduction in the free testosterone fraction. Presumably, such an effect in women would reduce the biologically available estradiol concentration, thus providing a mechanism by which a low-fat diet could favorably influence breast cancer risk and progression. Indeed, Prentice et al. (17), while observing no change in plasma SHBG, did find a decrease in the circulating bioavailable estradiol fraction when healthy postmenopausal women were placed on a diet providing only 20% of the total calories as fat. In the present study we did not observe any change in the serum SHBG levels when postmenopausal breast cancer patients, not receiving tamoxifen adjuvant therapy, were placed on a diet which contained approximately 34 g fat/day (20% of total calories). In keeping with these serum SHBG results, the low-fat diet had no detectable effect on the estradiol distribution in a small group of women who were not treated with tamoxifen. Although a more intensive reduction in fat intake might have altered the SHBG levels and estradiol distribution, it is noteworthy that Shimizu et al. (39) found no significant differences in the serum SHBG levels of American white postmenopausal women and postmenopausal Japanese women residing in rural areas where the diet typically contains approximately 10-11 g of fat/day (40).

Sakai et al. (7) determined both serum SHBG and CBG by competitive binding assays and found that both were elevated in advanced breast cancer patients treated with tamoxifen. In contrast to the present results, the increase in serum SHBG binding capacity was maximal within 3 weeks of commencing tamoxifen administration. The serum gonadotropin levels have been reported previously to be partially suppressed in tamoxifen-treated postmenopausal women (6, 14, 41), and their assay has been proposed as a simple indicator of patient compliance (6). We have confirmed this effect of tamoxifen, but the responses observed were quite variable, and a comparison of serum FSH, CBG, and SHBG indicates that the last provides the better indicator of tamoxifen activity.

ACKNOWLEDGMENTS

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