Effects of Adjuvant Chemohormonal Therapy on the Ovarian and Adrenal Function of Breast Cancer Patients

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ABSTRACT

Ovarian and adrenal function were studied in premenopausal breast cancer patients before and at intervals during adjuvant therapy with cyclophosphamide, methotrexate, and 5-fluorouracil (CMF), CMF plus prednisone (CMFP), or CMFP plus tamoxifen (CMFPT). Amenorrhea developed within 10 months of starting therapy in 13 of 15 patients given CMF, 8 of 10 receiving CMFP, and all of 13 CMFPT-treated patients. The amenorrheic patients receiving CMF showed a reduction in their plasma total estrogens and an increase in plasma luteinizing and follicle-stimulating hormones, indicating that these cytotoxic drugs directly suppressed ovarian function. Plasma androstenedione levels, which are derived equally from the ovaries and adrenals before the menopause, were also reduced. Plasma dehydroepiandrosterone sulfate, a steroid predominantly of adrenal origin, was unaffected. CMFPT-induced amenorrhea was associated with similar changes in the plasma estrogens and gonadotropins, without a change in the gonadotropin levels. Although the plasma sex hormone-binding capacity was increased during CMFPT therapy, there was only a small reduction in the percentage of free plasma estradiol, with the result that the level of circulating unbound estrogen was increased. The plasma estrogens declined, with a corresponding increase in gonadotropins, after the onset of CMFPT-induced amenorrhea.

INTRODUCTION

Treatment of premenopausal patients with Stage II breast cancer by adjuvant chemotherapy frequently causes amenorrhea due to ovarian suppression (9, 14, 17). Meakin et al. (12) reported that ovarian irradiation plus prednisone is more effective than radiation castration alone as adjuvant therapy for premenopausal patients. Similar results might be achieved if endocrine ablation were accomplished by using a combination of cytotoxic drugs plus prednisone.

In the present study, we compared the effects of adjuvant therapy with CMF alone versus CMFP or CMFPT on plasma androstenedione, DS, and total E1 + E2 levels. Plasma free E1 + E2 and SHBG assays were performed in patients treated with CMFPT to gain some insight into the significance of elevated plasma total E1 + E2 concentrations which occur in these women (15).

MATERIALS AND METHODS

Fifteen premenopausal patients received CMF, 10 were treated with CMFP, and 13 with CMFPT. Patients treated with CMF received cyclophosphamide (100 mg/sq m/day p.o. from the 1st to 14th day), methotrexate (40 mg/sq m i.v. on the 1st and 8th days), and 5-fluorouracil (600 mg/sq m i.v. also on the 1st and 8th days). The next cycle was started after a 2-week rest period (15th to 28th days), and subsequent cycles were administered at intervals of 4 weeks. The CMFP regimen consisted of the cyclical administration of CMF at 4-week intervals as described above, with the addition of prednisone (40 mg/sq m p.o. from the 1st to the 14th day of each cycle). The CMFPT combination consisted of CMF at 4-week intervals, plus prednisone (40 mg/sq m as before) and tamoxifen (10 mg p.o. twice daily throughout the entire period of adjuvant drug treatment). Drug doses with each of the 3 regimens were modified according to the severity of side effects.

The endocrine studies were performed immediately before therapy was commenced 4 to 6 weeks after mastectomy, after 4 weeks of treatment, and on alternate months thereafter. Blood samples were taken at 8 to 10 a.m., and the plasma was stored at -20° until analyzed. Plasma androstenedione, DS, E1 + E2, LH, and FSH were determined by radioimmunoassay (14) and free plasma E1 + E2 by equilibrium dialysis (19). Plasma SHBG capacity for estradiol was determined by competitive displacement of [3H]estradiol with increasing amounts of unlabeled hormone and Scatchard analysis (19). The assay had an intersay coefficient of variation of 6.4%.

The untransformed data are presented in the tables, but the log10 was used to compare steroid assay results between treatment groups by the unpaired Student’s t test. This was because the plasma concentrations are not distributed normally in a patient population but become so after logarithmic conversion. A paired Student’s t test was applied to evaluate the effect of therapy within the same groups.

RESULTS

Menstrual Cycles. Amenorrhea developed sometime within 10 months of starting therapy in 13 of 15 patients (average age, 42 ± 6 years) treated with CMF; one other had irregular menses; CMF, cyclophosphamide, methotrexate, 5-fluorouracil, and tamoxifen; CMFP, cyclophosphamide, methotrexate, and 5-fluorouracil; CMFPT, cyclophosphamide, methotrexate, 5-fluorouracil, and tamoxifen; DS, dehydroepiandrosterone sulfate; E1 + E2, estrone plus estradiol; SHBG, sex hormone-binding globulin; LH, luteinizing hormone; FSH, follicle-stimulating hormone.
menstrual periods and hot flashes. All but 2 of the 10 patients (average age, 41 ± 7 years) receiving CMFP became amenorrheic, and one developed irregular cycles with oligomenorrhea. Similarly, all 13 of the CMFPT-treated patients (average age, 38 ± 6 years) ceased to menstruate, including the 5 who were 35 years of age or younger. There was an inverse relationship between the patients' ages and the length of therapy required to produce amenorrhea (r = -0.605; p < 0.01; Chart 1).

Plasma Androstenedione and DS. The plasma androstenedione and DS concentrations before and 6 and 10 months after adjuvant therapy are summarized in Table 1. Patients treated with CMF showed a significant reduction in plasma androstenedione after 6 months (p < 0.01), but plasma DS levels were unaffected. In contrast, highly significant decreases occurred in the plasma DS concentrations of patients receiving CMFP (p < 0.001) or CMFPT (p < 0.01), as well as the plasma androstenedione (p < 0.001 and p < 0.01, respectively). The suppressive effect of prednisone on the adrenal secretion of androstenedione and DS was also evident when their plasma levels were compared in the 3 treatment categories (Table 1).

**Plasma Total E₁ + E₂, LH, and FSH.** The changes in plasma total E₁ + E₂, LH, and FSH concentrations of CMF and CMFP-treated patients were governed by the effects of therapy on menstrual activity. Eleven of the 13 CMF-treated patients who become amenorrheic did so within 5 months, and the group as a whole showed marked reductions in plasma total E₁ + E₂, with corresponding elevations in plasma LH and FSH, at 6 months (Table 2). Much greater variations were seen in those receiving CMFP because 5 of the 10 retained their menstrual cycles beyond 6 months of therapy, and 2 continued to have normal menses throughout their courses of treatment. The greater reduction in plasma androstenedione seen in the CMFP-treated patients who were amenorrheic at 10 months compared with the CMF-treated group did not influence the

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**Table 1**

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Pretherapy</th>
<th>6 mos.</th>
<th>10 mos.</th>
<th>Pretherapy</th>
<th>6 mos.</th>
<th>10 mos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMF (15)</td>
<td>136 ± 68b</td>
<td>92 ± 52cd</td>
<td>93 ± 33d</td>
<td>1.76 ± 0.84</td>
<td>1.83 ± 0.75</td>
<td>1.81 ± 0.94</td>
</tr>
<tr>
<td>CMFP (10)</td>
<td>123 ± 45</td>
<td>60 ± 41c</td>
<td>47 ± 18d</td>
<td>2.29 ± 0.88</td>
<td>1.05 ± 0.82c,d</td>
<td>0.98 ± 0.77c,d</td>
</tr>
<tr>
<td>CMFPT (12)</td>
<td>126 ± 46</td>
<td>66 ± 38c</td>
<td>47 ± 28d</td>
<td>1.96 ± 1.14</td>
<td>1.19 ± 1.26c</td>
<td>1.27 ± 0.98c</td>
</tr>
</tbody>
</table>

Numbers in parentheses, numbers of patients in each treatment group.

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**Table 2**

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Pretherapy</th>
<th>6 mos.</th>
<th>10 mos.</th>
<th>Pretherapy</th>
<th>6 mos.</th>
<th>10 mos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMF (15)</td>
<td>223±189b</td>
<td>75±82b</td>
<td>72±39b</td>
<td>19.0±8.3</td>
<td>120±82b</td>
<td>140±59b</td>
</tr>
<tr>
<td>CMFP (10)</td>
<td>235±131</td>
<td>206±169</td>
<td>123±88c</td>
<td>20.6±10.8</td>
<td>79±54c</td>
<td>147±106c</td>
</tr>
<tr>
<td>CMF ammenorhea (6)</td>
<td>235±145</td>
<td>219±189</td>
<td>96±36e</td>
<td>20.5±12.2</td>
<td>96±41e</td>
<td>181±89e</td>
</tr>
<tr>
<td>CMFPT (13)</td>
<td>164±86b</td>
<td>369±307</td>
<td>194±205</td>
<td>23.2±11.7</td>
<td>68±74</td>
<td>74±52c</td>
</tr>
</tbody>
</table>

Numbers in parentheses, numbers of patients in each treatment group.

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**Chart 1.** The relationship between age and length of treatment before the onset of amenorrhea for patients receiving CMF (•), CMFP (O), or CMFPT (□).

**Chart 2.** Changes in plasma E₁ + E₂ concentrations during treatment with CMFPT. □, increase in plasma E₁ + E₂ while the patients continued to have normal menstrual cycles; •, decline with the onset of amenorrhea.
Adjuvant Therapy and Endocrine Function

Thus, after 10 months of treatment, the mean plasma total E1 + E2 value in 14 women with disrupted menses due to CMF was 71 ± 41 pg/ml, while in the 8 CMFP-treated patients it was 96 ± 38 pg/ml.

The patients treated with CMFPT were of particular interest in that most showed an increase in plasma total E1 + E2 while they were having apparently normal menstrual cycles; with the onset of amenorrhea, there was a decline in the E1 + E2 levels (Table 2 and Chart 2). After 6 months of therapy, the 6 patients who were still cycling had a mean plasma total E1 + E2 of 694 ± 170 (S.D.) pg/ml, compared with a pretreatment mean of 141 ± 31 pg/ml (p < 0.01). These elevations were not accompanied by any significant change in plasma LH or FSH, the gonadotropins rising only with the onset of amenorrhea (Chart 3).

Table 3

<table>
<thead>
<tr>
<th>Time of Therapy</th>
<th>Plasma total E1 + E2 (pg/ml)</th>
<th>% of free E1 + E2</th>
<th>Calculated free E1 + E2 (pg/ml)</th>
<th>Plasma SHBG (nm/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretherapy</td>
<td>212 ± 94 a</td>
<td>3.06 ± 0.72</td>
<td>6.4 ± 3.0</td>
<td>107 ± 28</td>
</tr>
<tr>
<td>2-mo. therapy</td>
<td>454 ± 156 b</td>
<td>2.84 ± 0.68</td>
<td>12.0 ± 5.7 c</td>
<td>148 ± 38 d</td>
</tr>
<tr>
<td>3-mo. therapy</td>
<td>522 ± 104 b</td>
<td>2.73 ± 0.67</td>
<td>13.5 ± 3.5 b</td>
<td>152 ± 42 e</td>
</tr>
<tr>
<td>4-mo. therapy</td>
<td>389 ± 267</td>
<td>2.60 ± 0.41 f</td>
<td>11.5 ± 6.5 c</td>
<td>157 ± 61 c</td>
</tr>
</tbody>
</table>

a Mean ± S.D.
b Significantly different from pretherapy values (Student’s paired t test, p < 0.001.
c Significantly different from pretherapy values, p < 0.05.
d Significantly different from pretherapy values, p < 0.02.
e Significantly different from pretherapy values, p < 0.01.

DISCUSSION

This study confirms previous reports that ovarian function is frequently suppressed in premenopausal patients receiving adjuvant chemotherapy for breast cancer (9, 14, 17). We found there is an inverse correlation between age and the time to onset of drug-induced amenorrhea, a reflection, presumably, of increasing ovarian sensitivity to cytotoxic agents as a woman approaches her natural menopause. Our results also demonstrated that the inclusion of prednisone in the chemotherapeutic regimen suppresses adrenal steroidogenesis and that the inclusion of tamoxifen exerts a significant influence on ovarian function.

Androstenedione is produced both by the ovaries and by the adrenal glands. Before the menopause, the ovaries contribute approximately one-half of the circulating steroid (1). After the menopause, most of the plasma androstenedione is secreted by the adrenal cortex, although some secretion is maintained by the ovaries (6). The adrenal glands also contribute more than 90% of the circulating plasma DS in premenopausal women (1).

After adjuvant chemotherapy with CMF or with a single alkylating agent, the plasma androstenedione concentrations in premenopausal patients who develop amenorrhea are reduced to levels similar to those of untreated postmenopausal patients (14). There is also a small but significant fall in the plasma androstenedione concentrations in postmenopausal patients. However, the plasma DS concentrations are unaffected by adjuvant chemotherapy in either group of patients (14). These observations suggest that the altered plasma androstenedione concentrations after adjuvant chemotherapy are due to a loss of ovarian secretion, whereas the adrenal source of both androstenedione and DS remains intact.

Plasma Free E1 + E2 and SHBG Capacity. The mean percentage of free E1 + E2 for 10 patients prior to commencing CMFPT was 3.06 ± 0.72. A modest reduction, of marginal statistical significance (p < 0.05), occurred over the first 4 months of treatment, during which period prominent elevations in the plasma total E1 + E2 concentrations took place in most patients (Table 3). Plasma SHBG binding capacity for E1 + E2 showed small but consistent increases during CMFPT administration.

A patient who was not in the adjuvant therapy trial was studied before and during treatment with prednisone (40 mg daily) for radiation pneumonitis. The plasma SHBG capacity fell from 90 to 47 nm/liter after 5 weeks. After a period of dose reduction, steroid therapy was discontinued, and 3 months later the plasma SHBG capacity was 112 nm/liter.
The results of our present study demonstrate that the combination of cytotoxic chemotherapy plus prednisone (CMFP) reduces both the ovarian and the adrenal secretion of estrogen precursors. However, the plasma androstenedione assay is significant because it is much lower than the estrone estradiol production. In another study, Borkowski et al. (3) found that treatment of postmenopausal breast cancer patients with dexamethasone produced a rapid suppression of the plasma cortisol to undetectable levels, but measurable although reduced amounts of androstenedione remained in the plasma.

The administration of cytotoxic chemotherapy plus tamoxifen (CMFPT) increased the plasma E1 + E2 concentrations in premenopausal patients while they continued to have menstrual cycles. We suggested previously that tamoxifen might increase the plasma E1 + E2 concentrations in premenopausal women by blocking hypothalamic estrogen receptors, resulting in a failure of the normal feedback regulation of ovarian steroidogenesis by LH and FSH (13). Our present observation that high plasma E1 + E2 concentrations may occur without corresponding change in the gonadotropins following CMFPT administration does not necessarily negate this explanation. The plasma LH and FSH levels were not suppressed in the presence of increased circulating estrogens, and in normal premenopausal women receiving tamoxifen alone there is an exaggerated gonadotropin response to LH-releasing hormone stimulation although the basal levels are normal (7, 18).

Sakai et al. (16) reported that tamoxifen increases the plasma SHBG capacity for binding dihydrotestosterone, presumably because of a direct estrogen-like effect on the hepatic synthesis of SHBG. We used [3H]estradiol, which binds to SHBG to only one-third the extent of dihydrotestosterone (5), to demonstrate increased binding capacity during CMFPT therapy. However, increased plasma binding cannot account entirely for the elevated plasma E1 + E2 concentrations in our present study. Although there did appear to be some reduction in the percentage of plasma free E1 + E2 after 4 months of treatment with CMFPT, the increases in the total plasma E1 + E2 concentrations were relatively much greater. Therefore, the calculated absolute levels of circulating unbound hormone were also elevated. These results are supported by another study demonstrating that the administration of tamoxifen alone to normal premenopausal women results in an augmentation of the preovulatory increase in urinary estrone excretion (18). This would not occur if all the elevated plasma E1 + E2 was bound to SHBG.

One question of clinical importance is whether high plasma levels of physiologically active free E1 + E2 induced by tamoxifen can exert a deleterious effect in premenopausal patients with breast cancer. Lippman and Bolan (10) found that, although the presence of 10^-7 M tamoxifen inhibited the incorporation of [3H]thymidine into MCF-7 breast cancer cell cultures, the block was reversed when 10^-8 M estradiol was added to the medium. However, at the doses used clinically, the concentrations of tamoxifen achieved in the tumor are likely to be many times that of estradiol. In addition, there is evidence that compounds considered to be antiestrogens are, in fact, prodrugs which are metabolized in vivo to derivatives with much greater affinity for tumor estrogen receptors (8).

A second question of clinical importance is whether the decreased adrenal secretion of estrogen precursors which we observed following treatment with cytotoxic chemotherapy plus prednisone might exert a favorable influence on premenopausal patients with breast cancer. It is known that the peripheral aromatization of androstenedione provides essentially the sole source of circulating estrogens in postmenopausal or oophorectomized women. The elimination of this estrogen precursor accounts for the efficacy of adrenalectomy in postmenopausal patients with recurrent breast cancer (4). As noted previously, Meakin et al. (12) have reported that ovarian irradiation plus prednisone is more effective than radiation castration alone as adjuvant chemotherapy for premenopausal patients.

In this study, the decrease in plasma androstenedione concentrations in patients treated with CMFP had no effect on their plasma E1 + E2 concentrations. This result was not entirely unexpected, since an average of only 1.3% of [14C]androstenedione is converted to estrone by either intact or oophorectomized women (11). In order to have achieved a significant decrease in their plasma E1 + E2 concentrations as compared with patients treated with cytotoxic chemotherapy alone, our patients who received CMFP would have required a much greater decrease in their plasma androstenedione concentrations than we observed with the intermittent high-dose schedule of prednisone used in this study. It remains a matter of speculation as to whether or not the continuous daily schedule of prednisone used by Meakin et al. (12) would have been more effective in suppressing plasma androstenedione and E1 + E2 concentrations.

At present, two large cooperative groups are conducting randomized trials to evaluate the effects of intensive cytotoxic chemotherapy with and without prednisone in primary breast cancer. The Eastern Cooperative Oncology Group is using an intermittent high-dose schedule of prednisone similar to the one used in our study. The Ludwig Group is using a continuous low-dose schedule similar to the one used by Meakin et al. (12). The results of these prospective clinical trials should help to clarify the role of adrenal suppression in the adjuvant treatment of primary breast cancer.

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