Androgen-Estrogen Production Rates in Postmenopausal Women with Breast Cancer

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Abstract

In an attempt to define the hormonal environment of women with breast cancer, we examined the blood production rates of androstenedione and its contributory role as a prehormone of estrone in a group of 46 postmenopausal women with advanced metastatic breast cancer. Androstenedione blood production rates averaged 1.85 mg/day and were not significantly different from 9 agematched women with other neoplasms or from 4 noncancer controls. Similarly, blood production rates of estrone were not significantly different, averaging 56.9 μg/day in women with breast cancer compared with 43.9 and 41.8 μg/day in the control groups. The blood transfer constants (pK*p) were again similar in the breast cancer women versus the 2 control groups (2.13% versus 2.35 and 2.74%, respectively).

Despite the similar magnitude of androstenedione, estrone production rates, and transfer constants, androstenedione conversion to estrone accounted for only 72% of the total estrone production rate in women with breast cancer versus 92 and 95% in the 2 control groups. These differences were significant at P < 0.05.

As part of these studies, we noted that 14 of 46 women with breast cancer exhibited unusually high metabolic clearance rates of estrone ranging from 2600 to 5400 liters/day. These women could not be differentiated from other breast cancer patients on clinical grounds. Five patients had elevated clearance rates of androstenedione, but testosterone and estradiol clearance rates were not abnormal. Patients with the high metabolic clearance rates of estrone had lower plasma estrone concentrations so that blood production rates were similar to those noted in breast cancer patients with normal clearances. The differences in metabolic clearance rates were not associated with changes in the binding protein, sex hormone-binding globulin.

We concluded that androstenedione production rates and conversion to estrone are normal in postmenopausal women with breast cancer and that estrone production rates are normal in these women, yet that approximately 30% of estrone produced in postmenopausal women with breast cancer comes from source(s) other than prehormonal androstenedione.

Introduction

Although estrogenic hormones have long been suggested as playing a promoting role in the genesis of human breast cancer, previous studies of estrogen excretion and endogenous estrogen production rates in women with breast cancer have not shown consistent abnormalities (1, 2, 9, 11, 13, 15–18, 22, 24, 29, 32). In the current study we have explored both estrone production rates and the contributory role of its major prehormone, androstenedione, in a group of 46 postmenopausal women with advanced breast cancer. Whereas estrone production rates were not abnormal in women with breast cancer, a significant proportion of estrone produced in these patients arose from sources other than androstenedione.

Patients

Forty-six postmenopausal women with advanced metastatic breast cancer were studied before or between courses of systemic chemotherapy. All women had undergone natural menopause (no menses for 2 years) or had experienced surgically induced menopause prior to the discovery of their breast cancer. None of the patients had undergone endocrine-ablative surgery (except oophorectomy in the distant past for unrelated reasons), and no systemic endocrine therapy was used for at least 6 months prior to these studies.

Nine age-matched women with metastatic carcinomas originating from sites other than the breast served as cancer controls, and 4 postmenopausal women recuperating from non-cancer-related illnesses, excluding hepatic and cardiac disorders, served as noncancerous controls.

Informed consent was obtained from each patient prior to her entry into the study. The studies were performed between 8 and 10 a.m., in the fasting state, and before the patient arose from her overnight supine position.

Methods

MCR's. During the initial phase of this study, MCR's of androstenedione and estrone were determined sequentially. [3H]Androstenedione was infused at a rate of approximately 50 μCi/hr following a 10% loading dose given 30 min prior to the onset of the infusion. A Bowman constant infusion pump was calibrated to deliver approximately 1.5 to 1.8 ml of infusion solution per min. Blood samples were drawn distant to the infusing site at 60, 75, and 90 min. Immediately following the androstenedione infusion, a 10% loading dose of [3H]estrone was given and the second infusion of [3H]estrone (15 μCi/hr) was begun, with samples

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taken at 60, 75, and 90 min. These sequential infusion studies took approximately 4 hr.

Because of patient discomfort associated with the prolonged infusion times, subsequent studies were performed as a single infusion with a combination of 80 μCi [3H]androstenedione and 4 μCi of [14C]estrone. This latter procedure, taking approximately 120 min, was better tolerated by the patients. The blood samples obtained in lightly heparinized syringes were immediately centrifuged, and the plasma was stored at -15° for work-up at a later time.

Sample Purification. For the sequential studies, 10-ml portions of plasma taken at the various infusion times were used. Approximately 1500 cpm of [14C]androstenedione, [14C]testosterone, [14C]estrone, and [14C]estradiol were added to each plasma sample to monitor procedural losses and to serve as markers to determine the purity of the plasma extracts. For work-up of the [3H]estrone infusions, only [14C]estrone and [14C]estradiol were added to the plasma samples. To each tube were added 50 to 100 μg of unlabeled androstenedione, testosterone, estrone, and estradiol to simplify the recovery procedure.

Plasma samples were extracted 3 times with equal volumes of ether. The combined extracts were washed twice with 0.1 volume of water and dried. Separation of neutral from phenolic fractions was accomplished by toluene: 1 N NaOH partition, with the use of countercurrent procedure involving 3 sequential separatory funnels. The toluene layers following these partitions were combined, washed twice with 0.1 volume of water, dried over sodium sulfate, and filtered, and the extracts were dried. Preliminary purification of the samples was achieved by means of thin-layer chromatography with benzene:ethyl acetate (90:10). Testosterone fractions were separated from androstenedione fractions in this system. The testosterone and androstenedione fractions were separately eluted with ethyl acetate, and the dried eluates were acetylated with 10 drops of acetic acid and 5 drops of pyridine left overnight at room temperature. The acetates formed were further separated on the thin-layer system with benzene:ethyl acetate (90:10). The testosterone acetate and androstenedione fractions were purified further by forming methoxime derivatives by adding a few crystals of methoxymine hydrochloride and 3 drops of pyridine. The methoxime derivatives were chromatographed in the thin-layer system benzene:ethyl acetate (80:20). Aliquots (20%) of the eluates following each of the 3 thin-layer chromatography steps were taken for counting. Samples were considered “pure” when constancy of 3H:14C ratios were obtained (usually after the second thin-layer chromatography).

The phenolic fraction following the toluene:NaOH partition was neutralized to pH 5.5 with glacial acetic acid. The estrogens were extracted twice with equal volumes of ether, and the ether extracts were washed with 0.1 volume of water and dried. These fractions were further purified by formation of acetates as above, and separation of the acetates in the thin-layer system benzene:ethyl acetate (90:10). The dried thin-layer eluates were then saponified with 0.5 ml of 0.15 N NaOH in 80% methanol overnight, and the saponified estrogens were then chromatographed in the thin-layer system benzene:ethyl acetate (65:35). The estrone and estradiol fractions were then methylated, and the methyl ethers were purified on thin-layer chromatography with benzene:ethyl acetate (80:20). Appropriate aliquots following each purification step were taken for counting.

Radioactivity was determined in each sample with a Packard Model 3380 liquid scintillation counter. Samples were counted to accumulate approximately 10,000 3H and 14C counts. In general, each sample was counted for 50 to 100 min.

For the simultaneous infusion studies, approximately 1500 cpm of [14C]testosterone and [14C]androstenedione were added to each plasma sample prior to extraction. Since the estrogen fractions contained both 3H and 14C labels, approximately 500 μg of unlabeled estrone were used as a mass marker to monitor recovery. Samples were extracted and subsequently worked up as described above; however, for determination of estrone and estradiol recovery, samples were estimated for their mass with a Beckmann spectrophotometer, after the method of Longcope (C. Longcope, personal communication).

Plasma concentrations of androstenedione, testosterone, estrone, and estradiol were estimated from blood samples taken over a 10-min period prior to the onset of the infusion. Androstenedione and testosterone were measured by radioimmunoassay, as previously reported (12). Estrone was measured by radioimmunoassay according to the method of Longcope et al. (21). Estradiol was measured by a specific estradiol antibody provided by Dr. D. L. Loriaux. Preliminary purification of the estrogens was accomplished by using Sephadex LH-20 chromatography of the ether extract in each case.

Equilibrium dialysis of plasma samples was performed after the method of Chopra et al. (6) with a 1:5 dilution of plasma.

Results

Metabolic Clearance Rates

Estrogen. The mean MCR<sub>K</sub> for estrone, estradiol, and estrone was 2420 liters/day in our breast cancer patients, as noted in Chart 1. This value was significantly higher than the 1955 liters/day and 1820 liters/day noted in our non-breast cancer and noncancerous control patients, although the differences were not significant. On closer inspection of the MCRK<sub>E</sub> values, we were struck with several extraordinarily high values observed in some of our breast cancer patients. Indeed, 14 of the 46 women with breast cancer exhibited MCR<sub>K</sub> in excess of 2600 liters/day ranging up to 5400 liters/day. This group of women with extraordinarily high clearance rates of estrone was separately analyzed as Subgroup 2 (Table 1). They had a mean MCR<sub>K</sub> of 3550 liters/day, which was significantly higher than the mean MCR<sub>K</sub> of 1760 liters/day observed in the remaining 24 breast cancer women. Unfortunately, we could identify no unusual clinical features of the women with high MCR<sub>K</sub> versus those with normal MCR<sub>K</sub>. The ages of the women, duration and extent of metastatic disease, and drug history were similar for breast cancer patients with both high and low clearance rates. There was more...
Androgen-Estrogen Production Rates

**Blood Production Rates**

**Estrone.** The mean blood production rate of estrone \( (P_{bE}) \) was 56.9 \( \mu g/day \) in women with breast cancer compared with mean values of 43.9 and 41.8 \( \mu g/day \) in the control women. These differences were of borderline significance. The breast cancer women with high MCR\(_{E2} \) had a mean \( P_{bE} \) of 68 \( \mu g/day \), but this value was not significantly different from the mean of the remaining women with breast cancer. The group of women with high MCR\(_{E2} \) had lower plasma estrone concentrations; thus production rates of the both groups were of similar magnitude.

**Androstenedione.** The mean blood production rate for androstenedione in the women with breast cancer was 1.85 \( mg/day \), with no significant difference in production rates observed in Subgroups 1 versus 2. The values for \( P_{bA} \) were not different from those noted in women with other cancers or without cancer.

**Steroid Interconversions**

**Conversion of Androstenedione to Testosterone.** The blood conversion rate of androstenedione to testosterone averaged 7.81\% in breast cancer women versus 7.93 and 8.05 in the 2 groups of control women. These differences were not significant.

**Conversion of Estrone to Estradiol.** The conversion of estrone to estradiol was 7.51\% in women with breast cancer versus 6.88\% in the women with cancers at other sites. Only 2 values were available in the noncancerous controls, 10.3 and 4.2\%.

**Conversion of Androstenedione to Estrone.** The blood transfer constant \( (\rho) \) of androstenedione to estrone was determined from the expression:

\[
\rho_{bE^1}^A = \frac{C_{AE^1} \times MCR_{E1}}{MCR_{A1}}
\]

where \( C_{AE^1} \) is the conversion rate of infused tracers. In women with breast cancer the \( \rho_{bE^1}^A \) was 2.13\% compared with mean values of 2.35 and 2.74\% in the control groups. Within the group of breast cancer women, those with high metabolic clearance rates of estrone had somewhat higher \( \rho_{bE^1}^A \) values (2.34\%) compared with 1.96\% in those women with normal MCR\(_{E2} \). The differences between groups and within the breast cancer women were not statistically significant.

**Origin of Estrone**

The contributory role of androstenedione \( (\Delta) \) to the blood production rate of estrone \( (E_1) \) was determined in each woman from the expression:

\[
\text{Contribution } \Delta \text{ to } E_1 = P_{bE}^A \times \rho_{bE^1}^A \times P_{bE_1}^A
\]

values of 88 and 94 \( ng/100 \text{ ml} \) in the 2 groups of control patients. As noted above, the women with higher clearance rates of estrone had slightly lower values of plasma androstenedione averaging 81 \( ng/100 \text{ ml} \) versus 92 \( ng/100 \text{ ml} \) in the breast cancer patients with normal clearance rates.
Table 1
Metabolic clearance, blood levels, and production of androgens and estrogens

<table>
<thead>
<tr>
<th>Metabolic clearance rates (liters/day)</th>
<th>Plasma concentrations (ng/100 ml)</th>
<th>Blood production rates (µg/day)</th>
<th>Kinetic data</th>
<th>Dialyzable fractions (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCR&lt;sub&gt;1&lt;/sub&gt;</td>
<td>MCR&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Estrone</td>
<td>Androstenedione</td>
<td>Estradiol</td>
</tr>
<tr>
<td>Breast cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (32 women)</td>
<td>1760 ± 474&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>2130 ± 143</td>
<td>2.9 ± 1.2</td>
<td>92 ± 39</td>
</tr>
<tr>
<td>2 (14 women)</td>
<td>3550</td>
<td>2500</td>
<td>1.8</td>
<td>81</td>
</tr>
<tr>
<td>Overall</td>
<td>2420</td>
<td>2220</td>
<td>2.5</td>
<td>89</td>
</tr>
<tr>
<td>(46 women)</td>
<td>1085 ± 120</td>
<td>± 1.2</td>
<td>± 39</td>
<td>± 1.4</td>
</tr>
<tr>
<td>Non-breast cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1955</td>
<td>1960</td>
<td>2.4</td>
<td>88</td>
<td>2.9</td>
</tr>
<tr>
<td>(9 women)</td>
<td>429 ± 360</td>
<td>± 2.9</td>
<td>± 36</td>
<td>± 2.3</td>
</tr>
<tr>
<td>Noncancerous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1820</td>
<td>1890</td>
<td>2.2</td>
<td>94</td>
<td>2.8</td>
</tr>
<tr>
<td>(4 women)</td>
<td>211 ± 140</td>
<td>± 0.8</td>
<td>± 40</td>
<td>± 1.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± S.D.
<sup>b</sup> Differences significant at p < 0.05.
where \( P_{A} \) is the blood production rate of androstenedione, \( P_{E} \) is the blood production rate of estrone and \( k_{T} \) is the blood transfer constant. The values are displayed in Chart 2. In both groups of women with breast cancer (high MCREL and normal MCREL) 68 and 76% of their blood estrone production rate arose from peripheral metabolism of androstenedione. By contrast, in both control groups virtually all their estrone production rate arose from peripheral transformation of androstenedione. These differences were statistically significant at \( p < 0.05 \).

Dialyzable Fractions of Plasma Androgens and Estrogens

In view of the subgroup of breast cancer patients with unusually high clearance rates of estrone, we wished to examine the binding of various androgens and estrogens to their carrier protein, SHBG. We thus set up dialysis cells after the method of Chopra to determine the dialyzable fraction of testosterone, estrone, and estradiol as well as that of androstenedione (a control C19 androgen with no appreciable binding affinity for SHBG). These values are shown in Table 1. The dialyzable testosterone fraction was 2.80% in the breast cancer women versus 1.67 to 2.18 in the control groups, respectively. Within the breast cancer patients, those women with high MCREL exhibited slightly higher values, with a mean of 3.08% versus 2.68% in women with normal estrone clearance rates. These values were not statistically significant from each other. As for estrone binding affinity, breast cancer women exhibited slightly higher dialyzable fractions compared with the control groups, but again there was no difference in dialyzable fractions in those breast cancer patients with high clearance rates versus normal clearance rates for estrone. No significant differences in dialyzable fractions existed for estradiol or for androstenedione in the varying groups. It was thus apparent that the differences noted in metabolic clearance rates could not be explained by alterations in the sex hormone-binding protein.

Discussion

A large body of circumstantial evidence from the areas of epidemiology, experimental biology, and clinical medicine suggests that estrogens are important in the genesis of human breast cancer (13, 15–18, 22, 32). Indeed, if estrogen production and/or metabolism were found to be abnormal in women with breast cancer, these findings might be of great potential significance as a possible attack point for the prevention of the disease. Unfortunately, previous studies of estrogen production and urinary estrogen metabolites in women with breast cancer have been equivocal and conflicting. The epidemiological data of MacMahon et al. (23) and Dickinson et al. (7) showing differing ratios of urinary estriol versus estrone and estradiol in women at differing risks for breast cancer probably reflect differing pathways of estrogen metabolism, since estriol production rates were shown to be similar in women with high estriol ratios and low estriol ratios (20).

Over the past 2 decades Bulbrook et al. (3–5) reported that women with established breast cancer who responded poorly to endocrine therapy as well as women destined to develop breast cancer excreted lesser amounts of urinary 17-ketosteroid metabolites, androsterone, and etiocholanolone. Subsequently, Poortman (25, 26) and Thijssen et al. (30), reported lower production and excretion of dehydroepiandrosterone and its sulfate in women with breast cancer. These data suggesting a possible link between androgen production and metabolism versus breast cancer were unexpected since androgens were not thought to play an important role in the growth of breast tissue. It is now well appreciated that androgens serve as prehormones of estrogens (Chart 2). Indeed studies of Grodin et al. (8) and Longcope (19) and in our control patients have shown that virtually all estrogens made in postmenopausal women arise from prehormone metabolism to estrogens. We thus set out to test the hypothesis that women with breast cancer might metabolize androgens to estrogens in a greater degree than do normal women. This could result in greater production rates of estrogens, at levels still undetected by the less sensitive urinary estrogen excretion measurements.

If women with breast cancer excrete less 17-ketosteroid metabolites because they convert more C19 prehormones to estrogens, these findings would relate Bulbrook’s observations to abnormal estrogen production rates.

This exciting hypothesis was, unfortunately, not confirmed by the data in this study. We found that androstenedione and estrone production rates were no different in patients from androstenedione. See text for details. Bars, S.D.
women with breast cancer compared with 2 groups of control patients; also, the conversion of androstenedione to estrone was not abnormal in women with breast cancer. These data thus confirm earlier studies of Poortman et al. (25, 26) in this respect. Our studies showed, however, that conversion of androstenedione to estrone accounts for only 65 to 75% of the total blood production rate of estrone in women with breast cancer versus 92 and 95%, respectively, in the control groups. Thus, women with breast cancer appear to have an extra source of estrone production not noted in other postmenopausal women. Our studies do not shed light on whether a different prehormone is metabolized directly to estrone without mixing with the blood androstenedione pool or whether the estrone is secreted directly by the ovary or adrenals, or perhaps even produced within the malignant breast tissue.

Unexpectedly, we found a subgroup of 14 of 46 women with breast cancer who exhibited markedly elevated clearance rates of estrone and 5 women with increased clearance rates of androstenedione. This group of women had no specific clinically differentiating characteristics. Although there was more obesity in the women with high clearance rates, the magnitude of the obesity was not of the proportion reported by Schneider et al. (28) to be associated with high MCR's. Poortman (25) observed similar unusual MCR's in some of his breast cancer patients but made no further comment. In our studies, the higher MCR's, was associated with lower plasma levels of estrone so that estrone production rates were not appreciably changed. Similarly, the women with high clearance rates of estrone also exhibited slightly lower plasma concentrations of androstenedione and testosterone, as well as slightly different metabolic conversion rates of androstenedione to testosterone and estrone to estradiol. Thus plasma hormone concentrations and hormone metabolism seemed different in a subgroup of women with breast cancer.

Biologically active androgens and estrogens are bound in plasma to a carrier protein, SHBG. Since clearance rates of sex hormones are inversely related to protein binding (31), we examined the binding proteins in our patients with high versus normal clearance rates. No significant differences in binding characteristics for estrone, estradiol, or testosterone could be found between the breast cancer women with high versus normal clearance rates. Thus, the cause(s) of elevated estrone clearance rates in women with breast cancer remains unclear. In view of the high clearance rates noted in approximately 25% of breast cancer women, the significance of plasma hormone concentrations in women with this disease must be carefully considered.

The data presented in the current study do not support the hypothesis that women with established breast cancer make excessive amounts of estrogens from a prehormone pathway. Is it possible that our patient population, with established metastatic disease was not the optimal group to study? Henderson et al. (10) have shown that daughters of women with breast cancer (high risk) have higher plasma estradiol and prolactin levels (at comparable times of the menstrual cycle) than do other young women. It thus seems entirely possible that endogenous overproduction of estrogens via direct secretion or from prehormones might have occurred at a much earlier age and that with increasing age and debility, etc., these differences in estrogen production may have become blunted so that they are no longer discernible. It would seem that studies of prehormone metabolism in high-risk groups must be done before this attractive hypothesis is entirely abandoned.

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