Aromatase in Human Breast Carcinoma

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Abstract

Breast carcinoma tissue is capable of forming estrogens from circulating androgen precursors. In this study, aromatase was examined in homogenates of breast adipose and breast carcinoma tissue, in normal and abnormal parenchymal breast tissue, and in breast carcinoma cells in culture. Homogenates of carcinoma tissue showed a wide range of activity in the conversion of adrostenedione to estrone. The mean conversion in carcinoma tissue was greater than that seen in parenchymal tissue from patients with gynecomastia and mammary dysplasia. Homogenates of breast adipose tissue from patients with benign and malignant disorders showed comparable aromatase activity. Three cell lines isolated from a primary breast carcinoma differed in their aromatase activity demonstrating a heterogeneity of aromatase activity in cells from a single tumor. Studies of aromatase activity in breast carcinoma cells in culture over a period of 8 hr demonstrated progressive estrone formation.

Testosterone formation from androstenedione was noted in all studies using both homogenates and cell cultures. Testosterone formation from androstenedione was approximately 10-fold greater than was the formation of estrone from androstenedione in all studies. The metabolism of androstenedione to other androgens examined in homogenates of normal and carcinomatous breast tissue revealed that the major products were androsterone, 5α-androsterone, dihydrotestosterone, and epiandrosterone.

Both estrogen and androgen formation within the cell may be important in determining the cellular response.

Introduction

The ability of human breast carcinoma tissue to aromatize androgens to estrogens has been demonstrated by several groups (1–8). The role of this aromatization in the initiation, progression, or response to therapy has yet to be established. In addition, local metabolism of androstenedione and testosterone results in a series of reduced androgen metabolites which have the potential to exert biological effects which may be synergistic or antagonistic to the effects of estrogens.

Materials and Methods

The breast adipose and carcinoma tissues obtained at the time of surgery were homogenized and incubated under conditions described previously (11). In the incubations using tumor cells, the cells were homogenized prior to incubation to achieve conditions comparable to those used with whole tissue. The MD cell lines were prepared from a carcinoma of the right breast. The tumor was minced finely with scissors, and single cells were collected by passage of the supernatant through graduated needles. The single cells were suspended in 0.3% agar over 0.8% agar. The agar contained 15% fetal calf serum, crystalline insulin (0.2 units/ml), and amikacin (50 μg/ml). Colonies (>50 cells) developed after 14 days. Eight clones were picked and grown up in microwells, of which 5 survived for further study (MDA4, A4, A5, B1, and C).3 Cell lines A2, A5, and C were selected for the homogenate studies, and A2 and C were used for aromatase studies in culture. In all cases, following incubation, the reactions were terminated by extraction with ethyl acetate. [14C]Estrone, estradiol, testosterone, and dihydrotestosterone4 were added to correct for losses, and the residue was subjected to phenolic partition. The phenolic and neutral fractions were chromatographed separately, using thin-layer chromatography (benzene:methanol, 95:5). The fractions associated with estrone, estradiol, and testosterone were acetylated and rechromatographed as described previously (10). Radioactivity in the neutral fraction was also associated with dihydrotestosterone. Further purification of this fraction using thin-layer chromatography and recrystallization permitted the identification of [3H]androsterone, 5α-androsterone, dihydrotestosterone, and epiandrosterone.

Results

Homogenates of Breast Tissue. The results obtained when homogenates of a series of breast tumors were incubated with increasing concentrations of androstenedione are shown in Chart 1. The formation of estrone increased with increasing substrate concentration, and there was a tendency for estrone formation to plateau at substrate concentrations above 2 μM. These studies also show a wide variation in aromatase activity in different breast carcinomas, a finding which is consistent with our previous observations (9).

Studies designed to compare aromatase activity in breast adipose and breast carcinoma tissues are shown in Chart 2. The substrate concentration and product formation are expressed per 100 mg of tissue protein for purposes of this comparison. Estrone formation in adipose tissue from patients with breast carcinoma and from patients undergoing reduction mammoplasty for mammary dysplasia are shown along with estrone formation in carcinoma tissue. The pattern of estrone formation is similar in each tissue, but the amount of estrone formed at a given substrate concentration tends to be higher in carcinoma tissue. Chart 3 compares the mean estrone and testosterone formation in carcinoma and adipose tissue from 6 patients with breast carcinoma and in adipose tissue from 4 patients with mammary dysplasia at a substrate concentration of 1000 ng/100 mg of tissue protein. The formation of estrone and estradiol in adipose tissue is comparable from both sources. Estrone formation in carcinoma tissue tends to be higher, but there is a wide variation. Testosterone formation from androstenedione was comparable in the different tissues studied.


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3 M. E. Blackstein, manuscript in preparation.

4 The abbreviation used is: dihydrotestosterone, 5α-dihydrotestosterone.
patients with gynecomastia and mammary dysplasia was lower than in carcinoma tissue. The normal breast tissue obtained from an area remote from the carcinoma in patients undergoing mastectomy for breast carcinoma also had less estrone formation than did the carcinoma tissue, but this was not statistically significant due to the small numbers of normal incubations and the wide variation in the carcinoma tissues. The formation of estradiol was low in all tissues and could only be detected in one of six incubations with mammary dysplasia tissue and 4 of 5 incubations with gynecomastia tissue. The conversion of androstenedione to testosterone was approximately 10-fold greater than was the conversion of androstenedione to estrone in all tissues. The lowest testosterone formation was seen in

Chart 4 summarizes the studies comparing estrone, estradiol, and testosterone formation from androstenedione in parenchymal breast tissue. The added substrate concentration in these studies was low (0.03 to 0.2 µM) and, since the amount of endogenous substrate could significantly alter the specific activity of the added substrate, the results are expressed as percentage of conversion. Estrone formation in tissue from
mammary dysplasia and gynecomastia tissue.

**Cells from Pleural Effusions.** Cells derived from pleural effusions from 15 patients with breast carcinoma grown in monolayer culture were studied to determine their capacity for steroid metabolism. These cells were not cloned, represented mixtures of tumor cells as well as fibroblasts, and may have been affected by therapy prior to aspiration from the patient. One million cells were homogenized and incubated as described. Cells from all patients retained the ability to convert androstenedione to testosterone, but aromatization could only be demonstrated in 6 of 15 patients.

**Cultured Breast Carcinoma Cells.** The studies summarized in Charts 5 to 8 were carried out on cells derived from a patient (M. D.) with breast carcinoma. Clones MDA2, A4, and C were selected for metabolic studies. Increasing numbers of cells from 1 to \(32 \times 10^6\) were homogenized and incubated for 90 min. The formation of estrone from androstenedione (Chart 5) increased with increasing cell numbers in all cases. It is of interest that the clones of cells derived from the same tumor possess different aromatase activity. The formation of testosterone from androstenedione in these incubations is shown in Chart 6 and demonstrates that testosterone formation also increased with increasing cell number and that the clones differed in the formation of testosterone from androstenedione.

The aromatization of androstenedione to estrone and the conversion of androstenedione to testosterone were studied in 2 of these cell lines (A2 and C) in culture. After addition of \(^3H\)androstenedione to the culture medium, the medium was sampled at intervals for 8 hr. The formation of estrone (Chart 7) progressed linearly for the first 4 hr and appeared to be leveling off by 8 hr. Testosterone formation was linear over the 8 hr of incubation (Chart 8). It is interesting that the cell line which was least active in steroid metabolism in the homogenate (A2) was most active when the intact cells were studied in culture. The conditions under which these studies were carried out, however, were quite different, and factors such as the presence of fetal calf serum or the disruption of cell architecture may account for this observation.

**Androgen Formation.** In the experiments in which homogenates of breast carcinoma were incubated with \(^3H\)androstenedione, the neutral fraction contained some radioactivity which migrated with dihydrotestosterone on initial thin-layer chromatography. On further chromatography, 5β-androstenedione and epiandrostone were separated from androsterone, dihydro-

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**Table 1**

<table>
<thead>
<tr>
<th>Breasts Type</th>
<th>% of conversion of androstenedione to products (uncorrected losses)</th>
<th>(5β)-Androstan-3β-ol,17-one</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal breast</td>
<td>DHT 0.05 AND 1.16 (0.11^b)</td>
<td>0.71</td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td>0.13^a Epian-drostone 1.80 AND 0.05 0.70 0.01</td>
<td></td>
</tr>
</tbody>
</table>

^a Corrected for losses.
^b DHT, dihydrotestosterone; AND, androsterone; 5β-AND, 5β-androsterone.
testosterone, and 5β-androstane-3β-ol,17-one. Final identification of these metabolites was made by recrystallization to constant specific activity. A summary of the androgens identified in the "dihydrotestosterone fraction" following incubation with normal breast tissue and breast carcinoma tissue is shown in Table 1. The major component in this fraction was androstenedione followed by 5β-androsterone and dihydrotestosterone. The pattern of androgen formation was similar in normal and carcinoma tissue. This androgen formation takes place simultaneously with aromatization in these tissues. Each product may have a specific biological activity, and the net response of the cell may be the sum of these biological activities.

Summary

Breast carcinoma tissue from different patients has a wide range of aromatase activity. It is not known if this activity is a factor in the development or response of the tumor to hormonal manipulation. Cultures of cells from primary breast tumors retain the ability to aromatize androgens. Different clones of tumor cells from the same patient differ in their aromatase activity. Along with the conversion to estrogens, androstenedione is metabolized to testosterone and a series of 5α- and 5β-reduced products which may have specific biological effects. The biological response of the cell is a function of both estrogen and androgen formation.

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References

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