Adequacy of Estrogen Suppression with Aminoglutethimide and Hydrocortisone as Treatment of Human Breast Cancer: Correlation of Hormonal Data with Clinical Responses¹

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Abstract

Human breast neoplasms can be divided into hormone-dependent and hormone-independent subtypes. Estrogen is the major hormonal stimulus for growth of the dependent tumors. Failure to respond to estrogen suppression therapy could reflect either an incomplete lowering of estrogens or the hormonal independence of the tumor. To address this issue, we compared the levels of several estrogens and other hormones in women experiencing objective responses (the responders) and disease progression (the progression group) during therapy with the aromatase-steroidogenesis inhibitor, aminoglutethimide, and replacement hydrocortisone. Pretreatment hormonal profiles of the estrogens, androgens, ketosteroids, thyroxine, polypeptide hormones, and carcinoembryonic antigen did not differ significantly among response groups. During treatment, the levels of all estrogens were suppressed to a similar degree in the progression group and in the responders. Urinary estrone, for example, fell to 16.7 ± 3.2% of basal in the responders versus 16.3 ± 3.8% of basal in the progression group. These data suggested that lack of estrogen suppression did not explain the response to treatment in the patients receiving aminoglutethimide-hydrocortisone. This finding differs from our results in a similarly analyzed control group of patients treated with surgical adrenalectomy.

Levels of the weak androgens, dehydroepiandrosterone sulfate and androstenedione, were found to be higher in the progression group compared to the responders. This observation could not be explained by differences in duration of treatment between groups. Analysis at 1 to 12 weeks, 13 to 24 weeks, and 25 to 36 weeks after initiating treatment indicated higher androgen levels at each time point in the progression group. In addition, the results were not attributable to differing serum levels of aminoglutethimide among responder groups.

While the finding of higher androgen levels in the responder group remains unexplained, this study indicates that incomplete estrogen suppression is not responsible for lack of tumor response in patients with progressive disease during aminoglutethimide-hydrocortisone therapy.

Introduction

Hormone-dependent breast carcinomas respond to deprivation of biologically active estrogens with quantifiable tumor regression. AG, an aromatase-steroidogenesis inhibitor, in conjunction with replacement glucocorticoid, inhibits estrogen production. Failure to respond to such a regimen could indicate either an incomplete suppression of estrogens or lack of dependence of the tumor upon estrogens for growth. In an attempt to distinguish between these 2 possibilities, we compared the levels of various hormones during AG-HC therapy in 129 women with metastatic breast carcinoma. Patients were divided into those with objective tumor regression, stable disease, or progressive disease. The data indicated that incomplete suppression of estrogens does not explain the lack of tumor regression in patients treated with AG and HC.

Materials and Methods

Patients. Women with inoperable, recurrent, or metastatic carcinoma of the breast were treated with AG and HC (or dexamethasone) after obtaining their informed consent. Patients were excluded if greater than one-third of the liver was involved with metastatic disease, if central nervous system metastases were present, if they had progressive lymphangitic metastases to lung, or if survival was estimated to be less than 3 months. In the initial group of patients treated, the estrogen receptor status was not considered a criterion for entry, but later, known estrogen receptor-negative patients were excluded.

Response Criteria. Standard criteria were utilized to evaluate responses. CR required disappearance of all measurable lesions. PR included patients with a 50% or greater decrease in the sum of products of all measurable lesions with no new lesions appearing or with recalcification of lytic metastases in bone. These 2 groups of patients are collectively called the responders. Stable disease required a <25% increase or <50% decrease in the sum of products of all measurable lesions for at least 3 months with no new lesions appearing. Progressive disease included patients with a >25% increase in the sum of products of measurable lesions or the appearance of any new lesions during treatment. These patients are called the progression group.

Protocol of Therapy. All women received 250 mg of AG 4 times daily and 20 mg of HC at bed time, 10 mg every morning and 10 mg each evening at 5 p.m. during chronic therapy. During evolution of the protocol, twenty women initially received dexamethasone, 1 to 4 mg daily, before changing to HC.

Blood and Urine Collections. Twenty-four-hr urines were obtained on 3 to 5 consecutive days before starting therapy and at 2 to 12 weekly intervals thereafter. Blood samples were collected between 8 and 9 a.m. daily on the same schedule.

Hormone and Drug Assays. All hormones were measured by standard radioimmunoassay methods described previously in detail (6, 10, 11, 15, 17, 18). The polypeptide hormones were measured directly in serum or plasma. For the steroids, plasma or urine was first extracted, and radioimmunoassay methods described previously in detail (6, 10, 11, 15, 17, 18).

¹ Presented at the Conference "Aromatase: New Perspectives for Breast Cancer," December 6 to 9, 1981, Key Biscayne, Fla. Supported in part by National Cancer Institute Contract CS-NCI-53851 and Specialized Cancer Center Grant 1P30 CA18450 awarded by the National Cancer Institute, Department of Health, Education, and Welfare. This paper summarizes work presented in detail in an article published recently (19).

² The abbreviations used are: AG, aminoglutethimide; HC, hydrocortisone; CR, complete objective disease regression; PR, partial objective regression; DHEA-S, dehydroepiandrosterone sulfate; ACTH, adrenocorticotropic hormone.
then purified on Celite chromatography, and introduced into specific radioimmunoassays. Uretes required 48-hr incubation with Helix pomatise to cleave the glucuronide and sulfate bonds and release the respective free steroids. AG was measured with a colorimetric assay described previously (7).

**Statistical Analysis.** A matrix of data from each patient was entered into the Pennsylvania State University computer system. All pretreatment hormone measurements in individual patients were averaged. Values during treatment were then expressed as both absolute levels and percentages of the mean basal value for individual patients. Analysis of variance and paired and unpaired *t* tests with Neuman-Keul's correction for multiple comparisons were utilized where appropriate. Cumulative frequency distributions were constructed on data expressed as the mean of all values during treatment versus the basal level expressed as a percentage. Basal values were compared among all groups. For analysis of values during treatment, the responders (i.e., CR + PR) were combined, since the number of patients who experienced a complete tumor regression was small and since the stable group was omitted altogether.

**Results**

**Basal Hormone Levels**

For initial analysis, pretreatment hormonal profiles were compared among patients in each response category to determine whether any measurable parameter would predict the outcome on therapy. Data analysis included assessment of basal levels of estrogens, androgens, ketosteroids, thyroxine, polypeptide hormones, and carcinoembryonic antigen in women ultimately experiencing CR, PR, stable, or progressive responses to treatment (Table 1). No differences in the mean levels of estrogen, androgen, and ketosteroids were demonstrated prior to treatment among all groups. Of the 15 parameters measured, only follicle-stimulating-hormone differed significantly among groups by analysis of variance, and Neuman-Keul testing revealed that lower levels were observed in patients later progressing on AG-HC treatment. A trend (although nonsignificant statistically) toward higher basal levels of carcinoembryonic antigen [104 ± 49 ng/ml (n = 23)] in the progressive compared to the PR [14.8 ± 3.6 (n = 13)] and CR [25.5 ± 12 (n = 2)] groups was observed. A larger number of patients will be necessary to determine whether this difference is biologically significant.

### Chart 1

A, suppression of *P-E* (plasma estrone), *P-E$_2$* (plasma estradiol), and *E-S* (plasma estrone-sulfate) in women experiencing objective disease regression (CR-PR) or progression (Prog) in response to AG-glucocorticoid. **Blocks,** mean percentage of basal (pretreatment) values; bars, S.E. The numbers in the blocks indicate the number of patients for whom measurements were available. B, suppression of *U-E* (urinary estrone) and *U-E$_2$* (urinary estradiol). (Reproduced with the permission of the Annals of Internal Medicine.) NS, not significant.

**Table 1**

<table>
<thead>
<tr>
<th>Basal level</th>
<th>CR</th>
<th>PR</th>
<th>Stable disease</th>
<th>Disease progression</th>
<th>Analysis of variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>E$_1$ plasma</td>
<td>46.7 ± 11$^b$ (3)</td>
<td>51.4 ± 9.9 (20)</td>
<td>23.9 (1)</td>
<td>53.5 ± 9.4 (34)</td>
<td>0.94</td>
</tr>
<tr>
<td>E$_2$ plasma</td>
<td>13.0 ± 4.05 (3)</td>
<td>142 ± 1.43 (19)</td>
<td>11.6 (1)</td>
<td>12.4 ± 0.87 (35)</td>
<td>0.74</td>
</tr>
<tr>
<td>E$_S$ basal</td>
<td>566 ± 30 (5)</td>
<td>872 ± 211 (4)</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E$_1$ urine baseline</td>
<td>3.3 (1)</td>
<td>3.09 ± 0.86 (18)</td>
<td>4.63 ± 4.15 (2)</td>
<td>5.12 ± 1.34 (19)</td>
<td>0.65</td>
</tr>
<tr>
<td>E$_2$ urine baseline</td>
<td>0.73 (1)</td>
<td>0.59 ± 0.11 (18)</td>
<td>1.36 ± 0.87 (2)</td>
<td>0.94 ± 0.19 (18)</td>
<td>0.29</td>
</tr>
<tr>
<td>Aromatase index</td>
<td>0.0129 ± 0.0038 (3)</td>
<td>0.0246 ± 0.006 (18)</td>
<td>0.0208 ± 0.003 (30)</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>DHEA-S</td>
<td>1262 ± 551 (4)</td>
<td>634 ± 68 (34)</td>
<td>346 ± 113 (6)</td>
<td>713 ± 82 (59)</td>
<td>0.09</td>
</tr>
<tr>
<td>TSH</td>
<td>0.53 ± 0.06 (3)</td>
<td>0.74 ± 0.07 (31)</td>
<td>0.62 ± 0.23 (6)</td>
<td>0.79 ± 0.07 (54)</td>
<td>0.73</td>
</tr>
<tr>
<td>ACTH</td>
<td>8.2 ± 0.87 (3)</td>
<td>7.16 ± 0.33 (17)</td>
<td>6.61 ± 2.2 (4)</td>
<td>7.27 ± 0.41 (22)</td>
<td>0.73</td>
</tr>
<tr>
<td>TSH</td>
<td>4.01 ± 0.49 (3)</td>
<td>5.78 ± 1.66 (14)</td>
<td>6.44 ± 1.72 (3)</td>
<td>6.7 ± 1.41 (21)</td>
<td>0.95</td>
</tr>
<tr>
<td>LH</td>
<td>12.5 ± 0.41 (2)</td>
<td>17.1 ± 2.12 (10)</td>
<td>19.8 ± 6.2 (2)</td>
<td>26.7 ± 4.60 (17)</td>
<td>0.26</td>
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<tr>
<td>LH</td>
<td>524 ± 261 (2)</td>
<td>402 ± 57 (19)</td>
<td>286 ± 69 (4)</td>
<td>265 ± 37 (21)</td>
<td>0.11</td>
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<tr>
<td>FSH</td>
<td>2272 ± 1027 (2)</td>
<td>1551 ± 211 (19)</td>
<td>1584 ± 293 (4)</td>
<td>1303 ± 110 (22)</td>
<td>0.04</td>
</tr>
<tr>
<td>Aldosterone (urine)</td>
<td>0.72 ± 0.57 (2)</td>
<td>16.0 ± 3.2 (11)</td>
<td>20.0 (1)</td>
<td>16.2 ± 1.8 (19)</td>
<td>0.67</td>
</tr>
<tr>
<td>CEA</td>
<td>25.5 ± 12 (2)</td>
<td>14.8 ± 3.6 (13)</td>
<td>16.6 ± 7.7 (2)</td>
<td>104 ± 49 (23)</td>
<td>0.53</td>
</tr>
</tbody>
</table>

* E$_1$, plasma, estrone (pg/ml); E$_2$, plasma, estradiol (pg/ml); E$_S$, estrone-sulfate (pg/ml); E$_1$, urine, estrone (pg/24 hr); E$_2$, urine, estradiol (pg/24 hr); DHEA-S (ng/ml); TSH, thyrotropin-stimulating hormone (microunits/ml); ACTH (pg/ml); PRL, prolactin (ng/ml); LH, luteinizing hormone (ng/ml); FSH, follicle-stimulating hormone (ng/ml); CEA, carcinoembryonic antigen (ng/ml).

* Mean ± S.E.

$^b$ Numbers in parentheses, number in responder group.
Estrogen Suppression with AG and HC Treatment of Breast Cancer

Hormone Levels during Treatment

Estrogens. Data from all groups during treatment are expressed as a percentage of basal values to facilitate statistical comparisons. The levels of plasma and urinary estrone and estradiol and plasma estrone-sulfate did not differ between the responders and the progression group (Chart 1, A and B). Urinary estrone was suppressed to the greatest extent in both groups (i.e., 16% of basal) followed by plasma estrone-sulfate, plasma estrone, plasma estradiol, and urine estradiol.

To identify potential subgroups of patients within response categories, data were also analyzed by determining cumulative frequency distribution plots for each hormone (Chart 2, A to D). In the responders and the progression group, similar suppression curves for plasma and urinary estrone and estradiol were observed.

Androgens. In contrast to the estrogens, the progression group exhibited significantly higher levels of DHEA-S (p < 0.02) and of Δ4-androstenedione (p < 0.02) than did the responders (Chart 3). Cumulative frequency distribution plots (Chart 4, A and B) further accentuated these differences.

We questioned whether the higher levels of androgens observed in the progression group might merely reflect a shorter duration on AG-HC treatment. To address this issue, absolute levels of both steroids were analyzed prior to treatment and at 1 to 12, 13 to 24, and 25 to 36 weeks after initiating AG-HC therapy (Chart 5, A and B). At each time point, the levels of both androgens were higher in the progression group. Thus, there appears to be a basic difference between the responders and the progression group with respect to suppressibility on AG-HC.

AG Blood Levels during Treatment

The lack of suppression of androgens in patients progressing on therapy could reflect differences in the blood levels of AG
achieved. To assess this possibility, mean levels of AG were determined in patients in each response category. Serum AG concentrations did not differ among groups [CR, 9.8 ± 1.86 (n = 4); PR, 8.4 ± 0.94 (n = 22); stable, 5.67 ± 0.43 (n = 3); progression, 11.3 ± 1.37 µg/ml (n = 28)].

**Discussion**

Breast cancers can be divided into subgroups consisting of hormone-dependent and hormone-independent neoplasms (3, 5). Estrogen is currently considered to be the predominant hormonal stimulus of breast cancer growth in women. The failure of a patient to respond to hormone-suppressive therapy could indicate either a lack of adequate estrogen suppression or the hormone-independent nature of the tumor. Lack of sufficient estrogen suppression did not appear to be the explanation for tumor progression in patients treated with AG-HC, since the estrogens fell to the same extent (Charts 1 and 2) in nonresponders as in responders to therapy. Data from other clinical trials also support our conclusion that inadequate estrogen suppression with AG-HC was not a major reason for lack of response in the progression group. Newsome et al. (8) attempted to produce a further lowering of estrogens by surgical removal of the adrenals in patients first receiving AG-HC. No patients responded to the surgical adrenalectomy after first experiencing disease progression on AG-HC. Use of an antiestrogen to antagonize the biological action of the estrogens remaining during AG-HC treatment provides another means of addressing this question. Tamoxifen, when added to a regimen of AG-HC or given sequentially after it, appears to provide additional benefit in only a few patients (1, 2, 4, 14, 21). Taken collectively, these data suggest that AG-HC does suppress estrogen production to a biologically important degree in the majority of patients.

The observations regarding adequacy of estrogen suppression during AG-HC therapy differ somewhat from our findings in patients undergoing surgical adrenalectomy. When analyzed in an analogous fashion, patients progressing after surgical adrenalectomy have significantly higher levels of urinary estrone (p < 0.05; Chart 6) than objective responders. While not statistically significant, there is also a trend toward higher levels of plasma estrone and estradiol and urinary estradiol in the progression group. Furthermore, 2 of 4 patients given AG-HC after surgical adrenalectomy experienced an objective tumor regression, and all exhibited further suppression of estrogens (20). Thus, failure to respond to surgical adrenalectomy does indicate incomplete estrogen suppression in a subgroup of patients. This finding in the surgical adrenalectomy patients serves to emphasize the contrasting results in the patients treated with AG-HC.

We found greater levels of DHEA-S and androstenedione during AG-HC therapy in the progression group than in the
responders. Several possible explanations for this finding exist. The progression group may have been under greater stress from the metabolic effects of tumor progression and consequently had higher ACTH levels. A partial escape of the adrenal from inhibition (induced by ACTH) would cause increases in DHEA-S and androstenedione levels (9, 16). Under these circumstances, a concomitant rise in plasma and urinary estrogens would be prevented by the more distal aromatase block. ACTH measurements during therapy would serve to evaluate this possibility. Mean levels in the responder group (i.e., CR and PR) of 44 ± 6.5 pg/ml (n = 5) versus 73.1 ± 32 pg/ml in women (p not significant) with progressive disease (n = 5) would support this possibility. Further data are required, however, to establish the statistical significance of these preliminary observations.

It remains possible that biological differences exist between the responders and progression group which could explain the lack of DHEA-S and androstenedione suppression in the latter. Alternately, the persistent levels of androgen secretion in the progression group could stimulate the growth of androgen-dependent cells and causally explain their lack of tumor regression on therapy. This latter hypothesis would appear unlikely in light of the similar rates of response to surgical adrenalectomy versus AG-HC therapy. Androgen secretion is relatively preserved during AG-HC treatment, whereas surgical adrenalectomy marks a marked inhibition of androgen production (13). If androgen stimulation of tumor growth were occurring, one would expect lesser rates of response to AG-HC than to surgical adrenalectomy.

In summary, AG-HC appears to suppress the estrogens equally in the responders as in the progression group. This and other data suggest that the degree of estrogen suppression induced by AG-HC is sufficient to induce tumor regression in patients with hormone-dependent tumors.

Acknowledgments

The authors wish to thank Matilda Stover for her assistance in the care of patients entered into these studies and Marlene Thompson who provided excellent secretarial assistance in the preparation of this manuscript.

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