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Steroid Hormone Profiles in Women Treated with Aminoglutethimide for Metastatic Carcinoma of the Breast[1]

E. Samojlik, R. J. Santen, M. A. Kirschner, and N. H. Ertel

Department of Medicine, VA Medical Center, East Orange, New Jersey 07019 [E. S., N. H. E.]; Division of Endocrinology, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania 17033 [R. J. S.]; and Newark Beth Israel Medical Center, Newark, New Jersey 07112 [M. A. K.]

Abstract

Recent evidence suggests that aminoglutethimide (AG), a known inhibitor of adrenal steroidogenesis, is a potent blocker of aromatase and thus of estrogen production. These properties of AG have been exploited clinically to reduce the biosynthesis of adrenal estrogen precursors and extraglandular estrogen production in postmenopausal women with metastatic breast carcinoma.

In this study, we have explored the effects of AG on a variety of steroids, including Δ^5-C_{19} and -C_{21} compounds and Δ^4-C_{19} and -C_{21} steroids as well as plasma and urinary estrogens in a series of postmenopausal women with breast cancer treated for 2 to 26 weeks.

Plasma concentrations of Δ^5-C_{21} and -C_{19} compounds were reduced 3- to 5-fold during AG therapy and remained suppressed over the duration of the study. By contrast, the Δ^4-steroids such as progesterone, androstenedione, and 17α-hydroxyprogesterone rose 2- to 10-fold during the initial 2 weeks of AG treatment and then fell back to starting levels or were suppressed. Plasma levels of the potent androgens testosterone and dihydrotestosterone were relatively preserved during AG therapy. The possible contribution of the postmenopausal ovary to the above hormone levels during AG therapy was examined by comparing steroid values from surgically castrated and spontaneously menopausal women. No statistically significant differences between the two groups were observed.

In response to AG therapy, plasma levels of estrone and estrone sulfate were decreased 61 to 72%, and urinary estrone similarly fell 85% over the 12-week period. Estradiol concentrations in urine and plasma were similarly reduced 40 to 66% from basal values over this same period.

Introduction

Our group previously reported a method which uses AG,[2] an inhibitor of adrenal steroidogenesis, in combination with replacement glucocorticoids to treat postmenopausal women with hormone-dependent breast cancer (14, 16). AG blocks several cytochrome P-450-mediated steroid hydroxylation steps, including those required for conversion of cholesterol to pregnenolone and for aromatization of androgens to estrogens (3, 5–7, 18, 19, 21). While this drug regimen uniformly lowered plasma estrogen levels, initial studies suggested that Δ^4-A, a weak androgen, was paradoxically elevated during the early phases of treatment with AG and only variably suppressed later. This observation suggested that AG might alter the activity of certain intraadrenal enzyme pathways facilitating steroidogenesis. To further characterize the actions of AG, we systematically studied a variety of plasma steroids, including Δ^5- and Δ^4-C_{21} and -C_{19} compounds as well as estrogens in postmenopausal women with breast cancer undergoing "medical adrenalectomy."

Materials and Methods

Postmenopausal women with metastatic breast cancer received 1000 mg of AG daily in 4 divided doses as well as increasing amounts of dexamethasone or hydrocortisone (Charts 1 and 2). Blood and urine samples were collected prior to and during therapy, as described previously. The assays used for steroid measurements required extraction of the plasma and Celite column chromatography as preparatory steps prior to radioimmunoassay. The sensitivities, precision, and specificity of all assays used in this study have been described previously in detail (8, 10–13, 15, 16). Paired *t* tests were used for statistical analyses.

Results

Plasma Δ^5-Steroids. All Δ^5-steroids were suppressed from 3- to 5-fold during AG therapy. As shown in Chart 1, 17α-hydroxypregnenolone concentrations fell from basal levels 1.50 ± 0.07 (S.E.) to 0.37 ± 0.12 ng/ml during the first 2 weeks of treatment and continued to decline to 0.09 ± 0.02 ng/ml ($p < 0.001$) after 26 weeks of therapy. Plasma levels of DHEA sulfate decreased from basal values of 505 ± 89.3 to 129 ± 31.0 ng/ml ($p < 0.001$) after 2 weeks and later to 17.8 ± 5.3 ng/ml during chronic treatment. The pattern of DHEA suppression was similar to that of DHEA sulfate during chronic treatment.

Plasma Δ^4-Steroids. The pattern of Δ^4-steroids during AG therapy was strikingly different from those of the Δ^5-steroids. Plasma levels of progesterone, 17-Δ^4-P, and Δ^4-A became significantly elevated over basal values during the initial 2 weeks of therapy. As noted in Chart 2, plasma concentrations of 17-Δ^4-P rose 10-fold from basal levels of 0.65 ± 0.07 to 6.48 ± 1.46 ng/ml ($p < 0.01$) during 2 weeks of treatment and then fell to basal levels throughout therapy. The plasma values of progesterone and Δ^4-A also exhibited significant increments but of lesser magnitude (*i.e.*, 2- to 3-fold) than that of 17-Δ^4-P. Following their initial elevations, the levels of Δ^4-steroids then declined to pretreatment values during chronic therapy and then were suppressed further when hydrocortisone (40 mg/day) was substituted for dexamethasone. Only Δ^4-A decreased below basal levels of 0.57 ± 0.07 to 0.23 ± 0.05 ng/ml with chronic hydrocortisone ingestion at a dose of 40 mg/day ($p < 0.001$).

The plasma concentrations of 2 other Δ^4-steroids, namely, testosterone and DHT, were relatively preserved during AG

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[2] The abbreviations used are: AG, aminoglutethimide; Δ^4-A, androstenedione; DHEA, dehydroepiandrosterone; 17-Δ^4-P, 17-Δ^4-hydroxyprogesterone; DHT, dihydrotestosterone.

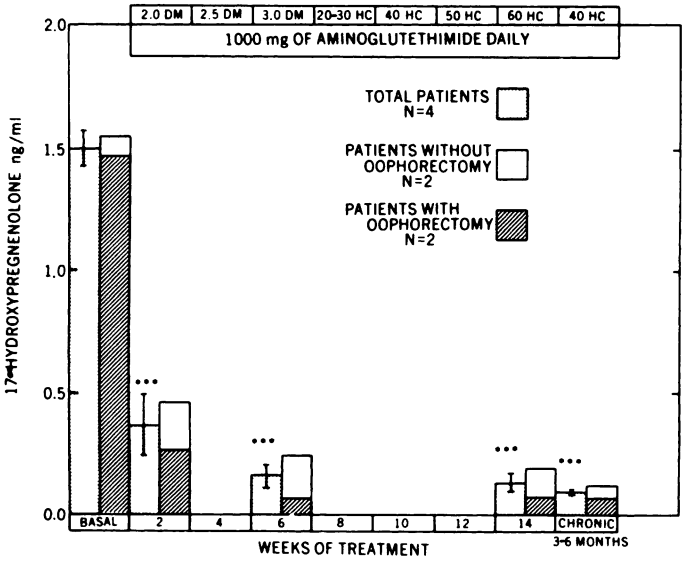

Chart 1. 17α-Hydroxypregnenolone levels during AG therapy. AG and either dexamethasone (*DM*) or hydrocortisone (*HC*) were administered in the daily dosages indicated. ▨, mean plasma concentrations of 17-Δ^5-P in 4 postmenopausal women with breast carcinoma before (baseline) and during 2, 6, 14, and 26 weeks of treatment with AG; *bars*, S.E. The second column group represents the separate values for 2 subgroups: □, mean levels in spontaneously postmenopausal women; ▨, surgically castrated patients. *Asterisks*, significant differences between basal and treatment values in the total group of patients (*, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$). *N*, number of patients.

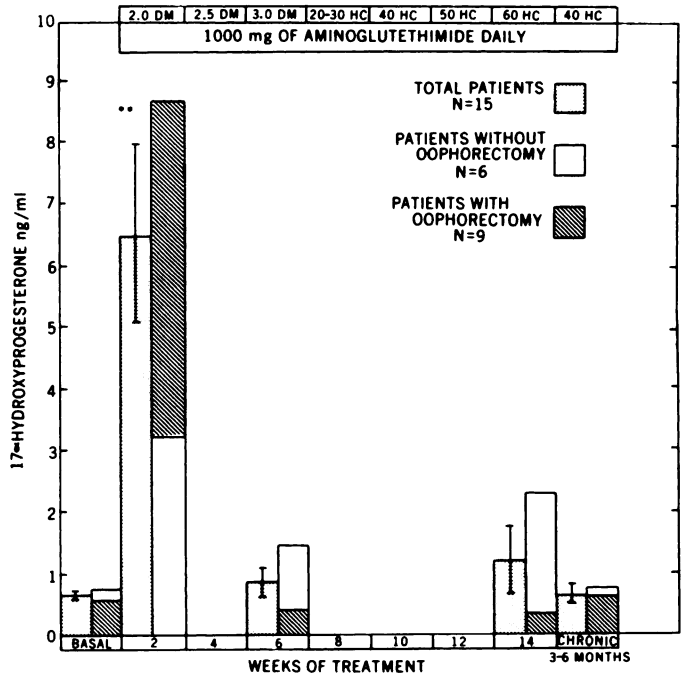

Chart 2. 17α-Hydroxyprogesterone levels during AG therapy. Abbreviations and symbols are as in Chart 1.

therapy. Testosterone levels changed only from 0.42 ± 0.06 to 0.34 ± 0.07 ng/ml (Chart 3) over the 12-week period of AG therapy. These differences were not significant. Similarly, plasma DHT concentrations exhibited minimal or no suppression during AG therapy (Chart 3).

Plasma and Urinary Estrogens. In response to AG treatment, the plasma and urinary levels of estrone fell 62 to 85%,

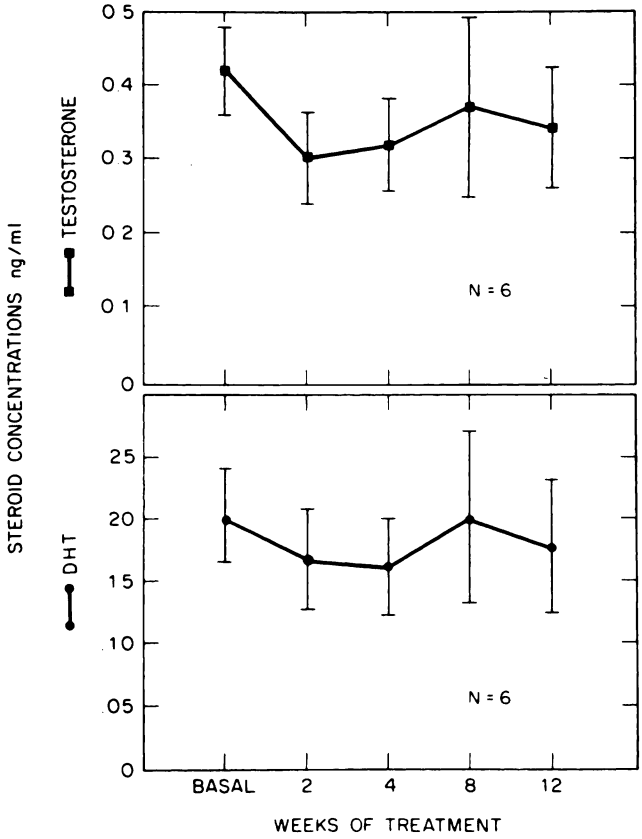

Chart 3. Plasma testosterone (■) and DHT (●) levels in women treated with AG. Data are mean for total group; *bars*, S.E. *N*, number of patients.

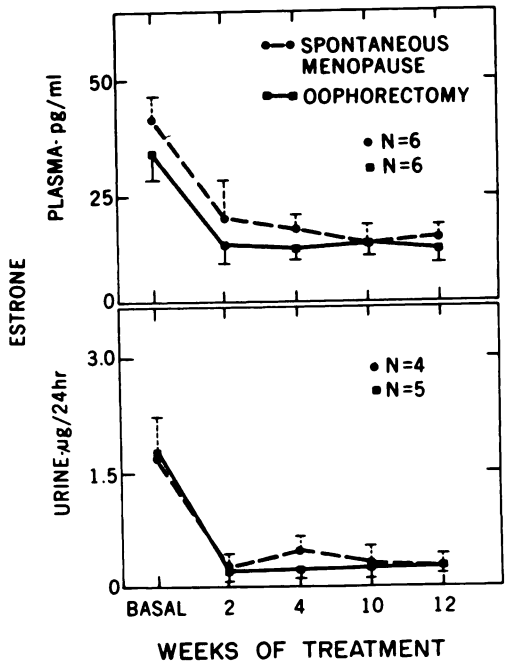

Chart 4. Plasma estrone levels in women treated with AG (*top*). Urinary estrone levels in women treated with AG (*bottom*). Data are mean for subgroups of women who are in spontaneous menopause (●) or surgical menopause (■); *bars*, S.E. *N*, the number of patients. No statistical significance between subgroups was observed.

respectively ($p < 0.001$), over the 12-week study period (Chart 4). Plasma estrone sulfate was decreased 72% by AG over the same period, as noted in Chart 5. Similarly, the concentrations of estradiol in both plasma and urine fell 40 to 66%, respectively, during the 12 weeks of AG therapy (Chart 6).

Steroid Profiles in Surgically Castrated *versus* Spontaneously Menopausal Women. To examine possible contributions of the postmenopausal ovary to the hormone levels measured during AG therapy, data from spontaneously menopausal *versus* surgically oophorectomized women were compared (Charts 1, 2, 4, 5, and 6). In both groups, the steady pattern of suppression of Δ^5-steroids and the biphasic rise and fall of Δ^4-steroids were observed. Furthermore, the pattern of estrogen responses to AG therapy was similar in both groups. Analysis of the pattern of each steroid in surgically castrated *versus* spontaneously menopausal women allowed tentative conclusions regarding the adrenal or ovarian origin of the changes observed. During the first 2 weeks of treatment, surgically castrated patients demonstrated slightly greater increases in progesterone (0.72 ± 0.25 ng/ml) over basal values than did spontaneously postmenopausal women. In addition, 17-Δ^4-P and 17α-hydroxypregnenolone plasma levels were greater in patients with intact ovaries. These findings suggested continued steroid secretion by the postmenopausal ovary. During AG therapy, the levels of the estrogenic steroids in surgically castrated subjects were not consistently lower than in spontaneously menopausal women (Charts 4 to 6).

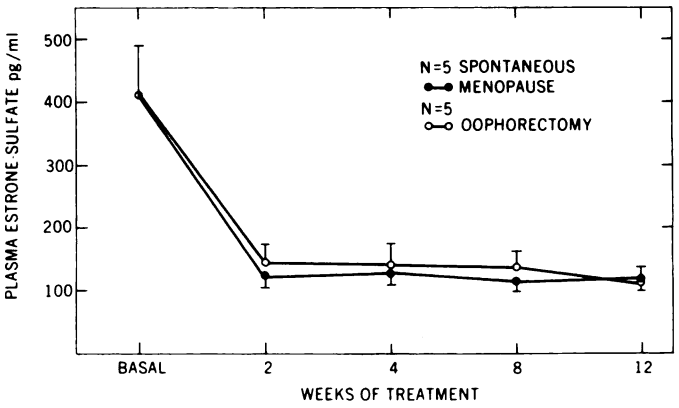

Chart 5. Plasma estrone sulfate levels in women treated with AG (medical adrenalectomy). Data are mean for subgroups of women who are in spontaneous menopause (●) or postoophorectomy (○); *bars*, S.E. *N*, number of patients. No statistical significance between subgroups was observed.

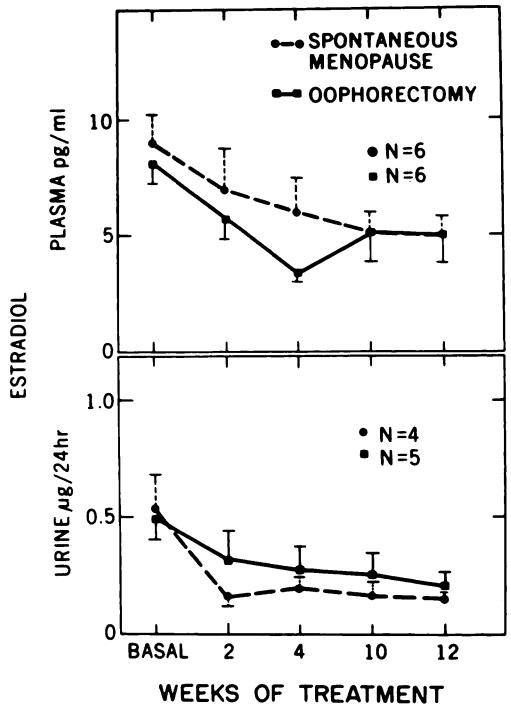

Chart 6. Plasma and urinary estradiol levels in women treated with AG. Abbreviations and symbols are as in Chart 4.

Discussion

As indicated in our previous studies, AG appears to be a potent inhibitor of adrenal steroid biosynthesis as well as a blocker of peripheral aromatization. AG binds to cytochrome P-450 complexes to block several steps in steroid hydroxylation (21). Initial studies demonstrated that AG inhibits conversion of cholesterol to pregnenolone by interfering with 20α-hydroxylation (6). Subsequent investigations revealed inhibitory effects on 3 hydroxylation steps necessary for the aromatization of androgens to estrogens. These properties of AG have been exploited clinically to reduce the synthesis of adrenal estrogen precursors and extraglandular estrogen production in postmenopausal women with metastatic breast carcinoma (15).

The current study demonstrated that AG paradoxically increased plasma levels of Δ^4-steroids, including progesterone, 17-Δ^4-P, and Δ^4-A during the initial 2 weeks of therapy and only later are these compounds restored to basal or suppressed levels. By contrast, Δ^5-steroids were markedly inhibited throughout AG therapy. This resulted in a reduction of the Δ^5-steroid:Δ^4-steroid ratios during AG therapy (10).

Decreased androgen production after AG therapy would be predicted if AG predominantly blocked conversion of cholesterol to pregnenolone, since this is a step required for androgen biosynthesis. However, the present study demonstrated preservation of testosterone and DHT levels during treatment with AG. Analysis of the known action of AG suggests a possible mechanism for the observed preservation of androgen secretion. AG in the doses used in this study (1000 mg daily) only partially inhibits the 20α-hydroxylation of cholesterol. As a reflection of incomplete inhibition, certain steroids requiring this step for synthesis such as 17-Δ^5-P, DHEA and DHEA sulfate were still measurable during drug administration, although at markedly reduced levels. The lack of suppression of Δ^4-steroids suggested the possibility that AG might enhance the conversion of Δ^5- to Δ^4-steroids by an alteration of the 3β-ol-dehydrogenase-Δ^5 to Δ^4-isomerase enzyme complex in the adrenal cells.

Other explanations for the pattern of Δ^5- to Δ^4-steroids are also possible. AG might reduce the metabolic clearance rate of Δ^4-steroids or perhaps increase the degradation of the Δ^5-steroids; however, when the metabolic clearance rate of Δ^4-A was measured before and during this therapeutic regimen, it was not altered (15). Furthermore, the metabolic clearance rates of the Δ^5-steroids would have to increase 3- to 5-fold to explain the differences observed, and this would seem unlikely.

The major evidence then suggests that AG produces pref-

erential conversion of Δ^5-steroid precursors to progesterone, 17-Δ^4-P, and Δ^4-A. Further metabolism to 11-deoxycortisol and cortisol is inhibited by the C-11 and C-21 hydroxylation blocking effects of AG as demonstrated by other investigators (17). Consequently, early steroid precursors that escape the blockade of 20α-hydroxylation could be preferentially shunted into the androgen pathway to be secreted as Δ^4-A, testosterone, and DHT. The observed elevations of Δ^4-steroids (progesterone, 17-Δ^4-P, and Δ^4-A) during AG therapy are also explained by this hypothesis (10, 12). In addition, the secretion of these steroids is further increased if glucocorticoid replacement is not given concomitantly with AG to prevent reflex adrenocorticotropic hormone increments. This mechanism produced the strikingly elevated levels of Δ^4-A reported by Newsome *et al.* (9) and could explain the earlier report of hirsutism occurring in women receiving AG without hydrocortisone supplementation (4).

The suppression of estrogen production with preservation of androgen levels in women with metastatic breast carcinoma might produce beneficial effects on tumor growth. Androgens have been used in the treatment of metastatic or inoperable breast carcinoma. In women with metastatic breast cancer who are fewer than 5 years postmenopausal and whose tumors are estrogen receptor positive, remissions may be induced by androgens with greater frequency than by estrogens. Women with metastatic breast carcinoma treated with antiestrogens in combination with androgens appear to experience tumor regression more frequently than do patients treated with antiestrogens alone (20). The mechanism by which androgens exert their effects on breast carcinomas and the amount of androgen required (*i.e.*, physiological *versus* pharmacological amounts) for tumor regression have not been fully clarified. It remains to be demonstrated, however, that the preservation of androgen secretion in our patients is of biological significance.

The data presented here demonstrate that AG inhibits adrenal biosynthesis and, to a lesser extent, ovarian secretion of steroid hormones at a number of steps. These current observations suggest a new action of AG, namely, the alteration of the 3β-ol-dehydrogenase-Δ^5- to Δ^4-isomerase complex in such a way as to favor the secretion of Δ^4-steroids. The combined effects of estrogen deprivation associated with androgen preservation might be significant in the therapeutic action of AG in hormone-responsive breast carcinoma.

Our data suggest that AG suppresses peripheral aromatase activity and markedly reduces estrogen concentrations in postmenopausal women with breast cancer. Although AG reduces adrenal secretion of Δ^5-C_{21} and -C_{19} compounds, there is an alteration in Δ^5- to Δ^4-isomerase complexes in such a way as to favor the secretion of the Δ^4-steroids. AG has little effect on the potent androgens testosterone and DHT.

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