Steroid Hormone Profiles in Women Treated with Aminoglutethimide for Metastatic Carcinoma of the Breast¹

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

Department of Medicine, VA Medical Center, East Orange, New Jersey 07019; Division of Endocrinology, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania 17033; 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 Δ⁵-C₁₆ and -C₂₁ compounds and Δ⁴-C₁₉ and -C₂₁ 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 Δ⁵-C₂₁ and -C₁₉ compounds were reduced 3- to 5-fold during AG therapy and remained suppressed over the duration of the study. By contrast, the Δ⁴-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 Δ⁴-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 Δ⁵- and Δ⁴-C₂₁, and -C₁₉ 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 Δ⁵-Steroids. All Δ⁵-steroids were suppressed from 3- to 5-fold during AG therapy. As shown in Chart 1, 17α-hydroxyprogrenenolone 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 Δ⁴-Steroids. The pattern of Δ⁴-steroids during AG therapy was strikingly different from those of the Δ⁵-steroids. Plasma levels of progesterone, 17α-Δ⁴-P, and Δ⁴-A became significantly elevated over basal values during the initial 2 weeks of therapy. As noted in Chart 2, plasma concentrations of 17-Δ⁴-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 levels of progesterone and Δ⁴-A also exhibited significant increments but of lesser magnitude (i.e., 2- to 3-fold) than that of 17-Δ⁴-P. Following their initial elevations, the levels of Δ⁴-steroids then declined to pretreatment values during chronic therapy and then were suppressed further when hydrocortisone (40 mg/day) was substituted for dexamethasone. Only Δ⁴-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 Δ⁴-steroids, namely, testosterone and DHT, were relatively preserved during AG...
therapy. Testosterone levels changed only from $0.42 \pm 0.06$ to $0.34 \pm 0.07$ 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%.
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 $\Delta^5$-steroids and the biphasic rise and fall of $\Delta^4$-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 ($0.72 \pm 0.25$ ng/ml) over basal values than did spontaneously postmenopausal women. In addition, $17\Delta^5$-P and $17\alpha$-hydroxyprogrenolone 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).

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 20a-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 $\Delta^4$-steroids, including progesterone, $17\Delta^4$-P, and $\Delta^4$-A during the initial 2 weeks of therapy and only later are these compounds restored to basal or suppressed levels. By contrast, $\Delta^5$-steroids were markedly inhibited throughout AG therapy. This resulted in a reduction of the $\Delta^5$-steroid:$\Delta^4$-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 20a-hydroxylation of cholesterol. As a reflection of incomplete inhibition, certain steroids requiring this step for synthesis such as $17\Delta^5$-P, DHEA and DHEA sulfate were still measurable during drug administration, although at markedly reduced levels. The lack of suppression of $\Delta^4$-steroids suggested the possibility that AG might enhance the conversion of $\Delta^5$- to $\Delta^4$-steroids by an alteration of the 3$\beta$-ol-dehydrogenase-$\Delta^5$ to $\Delta^4$-isomerase enzyme complex in the adrenal cells.

Other explanations for the pattern of $\Delta^5$- to $\Delta^4$-steroids are also possible. AG might reduce the metabolic clearance rate of $\Delta^4$-steroids or perhaps increase the degradation of the $\Delta^5$-steroids; however, when the metabolic clearance rate of $\Delta^4$-A was measured before and during this therapeutic regimen, it was not altered (15). Furthermore, the metabolic clearance rates of the $\Delta^5$-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 Δ⁴-steroid precursors to progesterone, 17-Δ⁴-P, and Δ⁴-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 Δ⁴-A, testosterone, and DHT. The observed elevations of Δ⁴-steroids (progesterone, 17-Δ⁴-P, and Δ⁴-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 adrenocorticotrophic hormone increments. This mechanism produced the strikingly elevated levels of Δ⁴-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 adrenomedullary 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-hydroxysteroid-Δ⁴- to Δ⁴-isomerase complex in such a way as to favor the secretion of Δ⁴-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 Δ⁴-C₂₁ and -C₁₉ compounds, there is an alteration in Δ⁴- to Δ⁴-isomerase complexes in such a way as to favor the secretion of the Δ⁴-steroids. AG has little effect on the potent androgens testosterone and DHT.

References

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


Cancer Res 1982;42:3349s-3352s.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/42/8_Supplement/3349s

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.