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Research Article

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Preservation of Androgen Secretion during Estrogen Suppression with Aminoglutethimide in the Treatment of Metastatic Breast Carcinoma

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ABSTRACT We evaluated the comparative effects of aminoglutethimide (AG) on androgen and estrogen levels estrone ([E_1], estradiol [E_2], plasma dehydroepiandrosterone-sulfate [DHEA-S], testosterone [T], dihydrotestosterone [DHT], Δ^4-androstenedione [Δ^4-A]), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin in postmenopausal patients with breast cancer randomly allocated to either AG treatment or bilateral surgical adrenalectomy as a control group. In response to either treatment, the plasma levels of E_1 fell 62–75% ($P < 0.001$) and urine E_1 85.7–88.7% ($P < 0.001$) in all study days over a 12-wk period. Similarly, the concentrations of E_2 in plasma and urine fell 40–72% without statistically significant differences between the two treatment modalities.

The relatively weak androgen, DHEA-S, was reduced by 92% (877.3±184.6 to 71.8±14.5 ng/ml) at 12 wk in women treated with AG, but suppressed nearly 99% (1,151±262 to 5.8±3.3 ng/ml) in adrenalectomized women. At all time points after treatment, the DHEA-S levels were significantly higher in patients receiving AG. Plasma concentrations of the potent androgens, T and DHT, were also relatively preserved during AG treatment. T levels were never significantly reduced by AG, and DHT concentrations were decreased only at the 4th wk to a maximum of 20%. Δ^4-A levels fell 56% in response to this drug only on the 12th wk of therapy (basal, 0.79±0.09 ng/ml; 12 wk, 0.35±0.07 ng/ml). In marked contrast, all androgens fell significantly at each time period in response to surgical adrenalectomy, with an 81% maximum suppression of T, 73% of DHT, and 97% of Δ^4-A. In response to estrogen suppression, plasma levels of FSH, LH, and prolactin did not change significantly throughout the treatment period in either therapy group.

To examine possible contributions of the postmenopausal ovary to hormone levels during therapy, data from surgically castrate and spontaneously menopausal women were evaluated separately. No significant differences between the two groups were observed for E_1, E_2, T, DHT, DHEA-S, Δ^4-A, LH, FSH, and prolactin.

We conclude that equivalent and highly significant estrogen suppression occurs with either AG or surgical adrenalectomy although androgen secretion is preserved during AG treatment but not after surgical adrenalectomy. The combined effects of estrogen deprivation associated with androgen preservation might be significant in the therapeutic action of AG in hormone-responsive neoplasms.

INTRODUCTION

30–40% of the unselected women with metastatic breast carcinoma and 50–60% with estrogen-receptor-positive tumors respond to surgical hypophysectomy, adrenalectomy, or oophorectomy with objective tumor regression (1–10). However, the considerable morbidity and significant mortality attendant upon these major surgical procedures limit their usefulness to specially selected patients. Consequently, a number of methods have been developed as possible non-

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surgical means to suppress pituitary or adrenal hormone production (11–15).

One of these techniques uses a pharmacologic inhibitor of adrenal steroidogenesis, aminoglutethimide, in combination with replacement glucocorticoid administration in postmenopausal or castrate women (14). The dual site of inhibitory action of aminoglutethimide on adrenal steroidogenesis and on the peripheral aromatization of androstenedione to estrone makes this compound highly potent in the blockade of estrogen production (16–19). Reduction of estrogen secretion is important because estrogen deprivation plays a major role in mediating the salutary effects of surgical oophorectomy or of hypophysectomy and adrenalectomy. However, androgens such as testosterone, dihydrotestosterone, and androstenedione can also influence the biologic growth rate of normal and neoplastic breast tissue (20). Other less active androgens such as dehydroepiandrosterone-sulfate may alter the binding of estradiol to receptors in mammary neoplastic tissue (21). In postmenopausal women, androgen administration by itself may ameliorate breast carcinoma growth (22).

Aminoglutethimide has been shown to alter certain intraadrenal enzyme pathways such as $\Delta^5$to Δ^4-isomerase-3β-ol dehydrogenase complex, the 11- and 21-hydroxylation reactions, and the conversion of cholesterol to pregnenolone (23–25). Because of these multiple effects, the androgen milieu in patients treated with aminoglutethimide could differ greatly from that produced by surgical removal of the adrenals or pituitary. During the performance of a controlled trial in women with breast carcinoma, we compared the levels of androgens and estrogens in patients randomized to either surgical adrenalectomy or aminoglutethimide treatment. The results demonstrate a highly significant suppression of estrogens in patients treated either medically or with surgical adrenalectomy. In marked contrast, androgen secretion continues during aminoglutethimide treatment but is markedly reduced by surgical adrenalectomy. Such preservation of androgen secretion associated with estrogen reduction could be an important aspect of the mechanism of action of aminoglutethimide.

METHODS

Steroids. Nonradioactive steroids were purchased from Steraloids Inc., Pawling, N. Y., and Sigma Chemical Co., St. Louis, Mo. [2,4,6,7-^{3}H]Estrone (E_1),[1] [6,7-^{3}H]estrone sulfate-ammonium salt, [2,4,6,7,16,17-^{3}H]17β-estradiol (E_2), [6,7-^{3}H]estradiol-17β-D-glucuronide, [1,2,4,5,6,7-^{3}H]dihydrotestosterone (DHT), [(*N*)-1,2,6,7-^{3}H]androstenedione (Δ^4-A), [(*N*)-7-^{3}H]testosterone (T), and [(*N*)-7-^{3}H]-dehydroepiandrosterone-sulfate ammonium salt (DHEA-S) were obtained from New England Nuclear (Boston, Mass.). Before use, the ^{3}H-labeled steroids were purified by celite microcolumn chromatography or thin-layer chromatography and stored as described by Manlimos and Abraham (26).

Hormone assays. A detailed description of the plasma E_1 and E_2 assays has been previously published (18). Briefly, these assays used ether extraction of 4 ml of plasma, celite chromatography, and then radioimmunoassay. The between-assay coefficients of variation are 14.7% for the E_1 and 14.8% for the E_2 assays. Within-assay coefficients of variation are 9.8% and 7.22%, respectively. For the E_1 assay, 3.7±0.3 (SEM) pg/tube produced a B/Bo of 90% and for the E_2 assay, 2.0±0.1 pg/tube was required. Antisera used for determination of Δ^4-A, DHT, T, E_1, and E_2 were gifts of Dr. D. Lynn Loriaux (National Institutes of Health, Bethesda, Md.). The antiserum used for measuring DHEA-S was purchased from the Radioassay Systems Laboratories, Inc., Carson, Calif.

The method for measuring E_1 and E_2 in urine used identical celite chromatography and radioimmunoassay as described for plasma estrogens, but after initial preparatory steps were carried out. Preextraction of an aliquot of urine with hexane (1:2 vol/vol) is performed to remove free steroids. An 0.2–0.5-ml aliquot of the hexane-extracted urine is then transferred into a glass tube containing 1.8–1.5 ml of 1 M acetate buffer, pH 5.0±0.1, 10,000 units of β-glucuronidase (Sigma Chemical Co.), 1,000 cpm of [6,7-^{3}H]estrone sulfate-ammonium salt, and 1,000 cpm of [6,7-^{3}H]estradiol-17β-D-glucuronide as recovery markers. The mixture set up in this manner is incubated for 48 h at 45°C in a dry bacteriologic incubator (Blue M Electric Co., Blue Island, Ill.). This urine is then extracted twice with 10 vol of absolute ethyl ether (MC/B; Metheson Coleman Bell, Norwood, Ohio), dried, taken up in isooctane, and applied to the celite columns. Percent recoveries for E_1 and E_2 are 82.3±5.6 and 78.7±7.4%, respectively. The blank in buffer samples prepared similarly to urine samples was 13.7±3.5 pg/tube for E_1 and 9.6±3.8 pg/tube for E_2 that was subtracted from each urine specimen during calculation. Within-assay and between-assay coefficients of variation for the E_1 assay are 4.3 and 9.8%, respectively, and 5.61 and 13.1% for the E_2 assay. The sensitivity of detection of E_1 and E_2 in 0.2-ml aliquots of urine is equivalent to 0.03 and 0.04 μg/24 h, respectively.

Radioimmunoassays of Δ^4-A, T, and DHT also use celite column chromatography and specific assays according to previously described methods. The sensitivities, recoveries, and coefficients of variation for these hormones were recently published (18, 23, 27).

DHEA-S levels were measured in plasma diluted 1:10–1:1,000 without extraction or further purification by the method of Buster and Abraham (28). Our modifications included substitution of phosphate buffer, pH 7.0, for diluent (0.1% of bovine γ-globulin in normal saline) and Δ^5-androsten-3β-ol-17-one sulfate sodium salt for free dehydroepiandrosterone (DHEA). After 2 h or overnight incubation of the reaction mixture, free steroid was separated from bound by dextran-coated charcoal. The supernate was poured into counting vials containing 10 ml of scintillation cocktail. After a 1-h preincubation at room temperature, all steroid samples were counted in a liquid scintillation counter (model LS 3133T; Beckman Instruments, Inc., Fullerton, Calif.) with 55% efficiency.

Serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were measured by a double antibody radioimmunoassay system as previously described (29), using

[1] *Abbreviations used in this paper*: AG, aminoglutethimide; Δ^4-A, androstenedione; DHEA, dehydroepiandrosterone; DHEA-S, DHEA-sulfate ammonium salt; DHT, dihydrotestosterone; E_1, estrone; E_2, 17β-estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; T, testosterone. These abbreviations are related to nonradioactive hormones. For radioactive hormones, see Methods.

reagents supplied by the National Pituitary Agency. With reagents supplied by the National Pituitary Agency, serum prolactin was also measured by radioimmunoassay according to the method of Hirvonen et al. (30).

Patients. After obtaining their informed consent, 25 postmenopausal or castrate patients were entered into a randomized trial of aminoglutethimide treatment (12 patients) vs. surgical adrenalectomy (13 patients) at The Milton S. Hershey Medical Center, Hershey, Pa. or Duke University Hospital, Durham, N. C. Women receiving aminoglutethimide were 64.6±3.3 yr of age and 66±4.7 kg mean wt compared with 57.9±1.9 yr and 64.9±2.8 kg in the surgical group. All women had metastatic breast carcinoma. In addition to these patients, we selected 8 normal cycling and 28 postmenopausal women to determine normal ranges and mean values of urinary estrogens (E_1 and E_2). We measured 56 samples in the follicular phase, 14 at mid-cycle, 56 in the luteal phase, and 65 in postmenopausal volunteers.

Protocol. Postmenopausal women under 70 yr of age were admitted to the study provided that metastatic tumor contained estrogen receptor concentrations >3 fmol/mg cytosol protein or that metastatic tumor tissue was not accessible for biopsy (estrogen receptor-unknown group). Those with tumors containing estrogen receptor concentrations of <3 fmol/mg cytosol protein were excluded. Women were then stratified into estrogen-receptor positive or estrogen receptor-unknown groups and into categories according to site of dominant disease (i.e., soft tissue, bone, or parenchymal involvement) and disease free interval (i.e., <2 or >2 yr). After stratification, patients were randomly assigned into either medical or surgical treatment groups.

The patients were admitted to the hospital for pretreatment studies. Blood samples were collected between 8 and 9 a.m. for 3 consecutive days for hormone assays; three 24-h urine samples were obtained over the same period. Patients then underwent surgical adrenalectomy or were started on 250 mg of aminoglutethimide four times daily. In the surgical group, preoperative and postoperative steroid coverage was administered to individual patients according to standard procedures. However, the dosage of hydrocortisone was not tapered below 100 mg daily (in divided doses) until 2 wk postoperatively. Patients randomized to aminoglutethimide similarly received 100 mg of hydrocortisone daily for the first 2 wk of therapy. Thereafter, both groups of patients were given 20 mg of hydrocortisone at the hour of sleep, 10 mg at 8 a.m., and 10 mg at 4 p.m. Mineralcorticoid replacement with 9α-fluorohydrocortisone was administered only if required, as detected clinically by orthostatic hypotension, abnormal plasma electrolytes, or symptoms suggestive of saline deficiency. Women were then followed in the Endocrinology Clinic every other week for the first 3 mo and monthly thereafter. Blood samples were collected between 8 and 9 a.m. and urine samples 24 h before each clinic visit. Standard staging procedures were carried out to assess clinical responses. All steroid and protein hormone determinations were performed on blood samples obtained basally (pretreatment), and at 2, 4, 8, and 12 wk for T, DHT, FSH, LH, and prolactin, and at 2, 4, 10, and 12 wk for the estrogens, Δ^4-A and DHEA-S. This change was necessary because of limited sample availability on weeks 8 and 10. In selected instances, as shown on Tables I–III, missing data are indicated by a reduced number of patients (n) for specific time points.

TABLE I

Comparison of Estrogen Levels between Patients Treated with Adrenalectomy or AG

Hormone determined	Basal levels		2 wk		4 wk		10 wk		12 wk	
	M*	S‡	M	S	M	S	M	S	M	S
E_1-plasma										
pg/ml	38.1	42.3	17.8	9.7	15.6	9.0	15.2	11.2	14.8	10.8
SEM	±3.8	±5.2	±4.0	±1.2	±1.7	±1.0	±1.3	±1.1	±1.8	±1.8
n§	12	13	12	13	12	13	12	13	12	13
Basal vs. Rx‖			$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
M vs. S			NS		$P < 0.01$		NS		NS	
E_1-urine										
μg/24 h	*1.75*	*3.10*	*0.24*	*0.46*	*0.29*	*0.40*	*0.25*	*0.44*	*0.26*	0.37
SEM	±0.30	±0.71	±0.06	±0.15	±0.09	±0.13	±0.07	±0.08	±0.05	±0.08
n	9	12	9	10	9	11	9	12	9	11
Basal vs. Rx			$P < 0.001$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.001$	$P < 0.01$	$P < 0.001$	$P < 0.01$
M vs. S			NS		NS		NS		NS	
E_2-plasma										
pg/ml	8.6	11.4	6.3	4.8	4.7	4.8	5.2	5.2	5.1	5.3
SEM	±0.7	±1.7	±0.9	±0.6	±0.8	±0.4	±0.7	±0.4	±0.6	±0.5
n	12	13	12	13	12	13	12	13	12	13
Basal vs. Rx			$P < 0.01$	$P < 0.01$	$P < 0.001$	$P < 0.01$	$P < 0.001$	$P < 0.01$	$P < 0.001$	$P < 0.01$
M vs. S			NS		NS		NS		NS	
E_2-urine										
μg/24 h	0.50	0.71	0.24	0.28	0.24	0.24	0.21	0.20	0.17	0.20
SEM	±0.07	±0.13	±0.06	±0.05	±0.05	±0.05	±0.04	±0.03	±0.02	±0.03
n	9	12	9	10	9	11	9	12	9	11
Basal vs. Rx			$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$
M vs. S			NS		NS		NS		NS	

* Medical treatment with AG.

‡ Surgical adrenalectomy.

§ Number of subjects.

‖ Statistical significance.

TABLE II

Comparison of Androgen Levels between Patients Treated with Adrenalectomy or AG

Hormone determined	Basal levels		2 wk		4 wk		8 wk§		12 wk	
	M*	S‡	M	S	M	S	M	S	M	S
DHEA-S										
ng/ml	877.3	1,151	182.2	14.0	148.5	30.0	74.7	3.3	71.8	5.8
SEM	±184.6	±262	±55.3	±4.3	±84.7	±23.9	±21.4	±1.6	±14.5	±3.3
n	12	13	11	12	9	11	12	12	12	13
Basal vs. Rx			$P < 0.01$	$P < 0.001$	$P < 0.01$	$P < 0.001$	$P < 0.01$	$P < 0.001$	$P < 0.001$	$P < 0.001$
M vs. S			$P < 0.01$		NS		$P < 0.01$		$P < 0.001$	
T										
ng/ml	0.42	0.43	0.28	0.13	0.32	0.08	0.37	0.11	0.34	0.08
SEM	±0.06	±0.06	±0.06	±0.06	±0.06	±0.009	±0.13	±0.02	±0.07	±0.02
n‖	6	6	6	6	6	6	6	6	6	6
Basal vs. Rx¶			NS	$P < 0.001$	NS	$P < 0.01$	NS	$P < 0.01$	NS	$P < 0.01$
M vs. S			NS		$P < 0.02$		NS		$P < 0.05$	
DHT										
ng/ml	0.20	0.15	0.16	0.05	0.16	0.03	0.20	0.03	0.18	0.04
SEM	±0.04	±0.02	±0.04	±0.01	±0.04	±0.006	±0.07	±0.007	±0.05	±0.01
n	6	6	6	6	6	6	6	6	6	6
Basal vs. Rx			NS	$P < 0.01$	$P < 0.02$	$P < 0.001$	NS	$P < 0.001$	NS	$P < 0.01$
M vs. S			$P < 0.05$		$P < 0.05$		NS		$P < 0.05$	
Δ^4-A										
ng/ml	0.79	0.96	0.47	0.07	0.54	0.03	0.63	0.02	0.35	0.03
SEM	±0.09	±0.14	±0.20	±0.04	±0.17	±0.01	±0.16	±0.007	±0.07	±0.01
n	12	13	10	13	11	10	12	13	11	13
Basal vs. Rx			NS	$P < 0.001$	NS	$P < 0.001$	NS	$P < 0.001$	$P < 0.01$	$P < 0.001$
M vs. S			$P < 0.05$		$P < 0.01$		$P < 0.001$		$P < 0.001$	

* Medical treatment with AG.
‡ Surgical adrenalectomy.
§ Δ^4-A and DHT = 10 wk.
‖ Number of subjects.
¶ Statistical significance.

TABLE III

Comparison of Gonadotropin and Prolactin Levels between Patients Treated with Adrenalectomy or AG

Hormone determined	Basal levels		2 wk		4 wk		8 wk		12 wk	
	M*	S‡	M	S	M	S	M	S	M	S
FSH										
ng/ml	1,667	1,818	1,800	1,900	1,481	2,041	1,736	2,070	1,911	2,107
SEM	±169	±136	±171	±197	±172	±207	±132	±155	±159	±173
n§	11	13	11	8	8	8	11	12	9	9
Basal vs. Rx‖			NS	NS	NS	NS	NS	NS	NS	NS
M vs. S			NS		NS		NS		NS	
LH										
ng/ml	452	355	432	383	433	399	387	393	357	369
SEM	±71	±51	±68	±130	±78	±95	±70	±76	±57	±64
n	11	11	11	4	8	10	11	11	7	11
Basal vs. Rx			NS	NS	NS	NS	NS	NS	NS	NS
M vs. S			NS		NS		NS		NS	
Prolactin										
ng/ml	14.4	15.9	8.8	23.4	27.9	23.8	14.2	12.7	18.3	18.7
SEM	±2.3	±3.1	±2.1	±9.0	±14.4	±7.6	±3.3	±1.7	±6.3	±7.1
n	10	12	9	6	8	9	10	9	7	8
Basal vs. Rx			NS	NS	NS	NS	NS	NS	NS	NS
M vs. S			NS		NS		NS		NS	

* Medical treatment with AG.
‡ Surgical adrenalectomy.
§ Number of subjects.
‖ Statistical significance.

Statistical analysis. The *t* test was used to compare hormone levels between patients treated with the two modalities and to evaluate the statistical significance of the degree of suppression of various hormone levels when compared with basal levels. An analysis of variance was applied when multiple comparisons were required. A computer-programmed analysis of covariance was used to evaluate statistical differences between oophorectomized and spontaneously menopausal patients.

RESULTS

Plasma and urinary E_1 and E_2. The mean values and absolute ranges of urinary E_1 and E_2 for normal cycling and healthy postmenopausal women were found to be similar to the quantities reported by others as shown in Table IV (31). Basal plasma E_1 levels were similar in patients randomized to aminoglutethimide (AG) treatment (38.1±3.8 pg/ml SEM) or to surgical adrenalectomy (42.3±5.2 pg/ml). In response to either treatment, the plasma levels of this steroid fell 62–75% ($P < 0.001$) on all study days over a 12-wk period (Table I). Although mean E_1 concentrations were slightly higher during treatment in the group receiving AG, this difference was significant only in the 4th wk ($P < 0.01$). In breast cancer patients, basal urinary levels of E_1 were not statistically different between women randomized to surgical adrenalectomy or AG therapy (3.10±0.71 μg/24 h, surgical vs. 1.75±0.30 μg/24 h, AG). After either form of treatment, the urinary excretion of this hormone decreased significantly by 85–88% (Table I) and both groups exhibited a similar degree of suppression.

E_2 levels in the blood and urine generally paralleled those of E_1 (Table I). Basal concentrations were similar in patients randomized to either treatment modality (plasma E_2: medical 8.6±0.7, surgical 11.4±1.7 pg/ml; urine E_2: medical 0.50±0.07, surgical 0.71±0.13 μg/24 h). In both groups, plasma and urinary E_2 levels fell ~40–72% and no statistically significant differences were observed between the patients treated with AG or surgical adrenalectomy.

Plasma androgen levels. As shown in Table II, the relatively weak Δ^5-androgen, DHEA-S, was suppressed significantly in both patient groups from similar basal values (medical 877.3±184.6, surgical 1,151±262 ng/ml). However, although the patients given AG exhibited a 92% maximum inhibition to 71.8±14.5 ng/ml by 12 wk, those undergoing adrenalectomy demonstrated a nearly 99% decrease to 5.8±3.3 ng/ml. At all time periods after treatment, the DHEA-S levels in patients receiving AG were significantly higher than those in the surgical group (Table II).

Even greater differences between medical and surgical treatment emerged when the Δ^4-steroids were examined systematically. The levels of T, DHT, and Δ^4-A were only variably suppressed by AG. T levels were never significantly inhibited by AG, and plasma DHT concentrations were suppressed only variably (i.e., the 4th wk) to a maximum of 20% (Table II). Δ^4-A fell in response to this drug only on the 12th wk of therapy (basal 0.79±0.09 ng/ml; 12th wk: 0.35±0.07 ng/ml, $P < 0.01$, Table II). In marked contrast, all Δ^4-androgens fell significantly at each time period (Table II) in response to surgical adrenalectomy. The maximum suppression of T was 81%, of DHT 73%, and of Δ^4-A 97%.

Plasma levels of FSH, LH, and prolactin did not change significantly throughout the treatment period in response to either form of therapy (Table III). The two groups did not differ at any time point.

Spontaneously menopausal vs. surgically castrate patients. To examine possible contributions of the

TABLE IV
Urinary Estrogen Excretion in Normal Cycling and Postmenopausal Women (μg/24 h)

Subjects	Hormone measured			
	E_1±SE	Range	E_2±SE	Range
Normal females				
Follicular phase	6.40±0.21 (56)	4.1–8.0	2.15±0.05 (56)	1.9–2.5
Mid-cycle	18.60±0.79 (14)	12.80–28.10	8.16±0.47 (14)	4.4–14.5
Luteal phase	10.50±0.77 (56)	5.7–14.8	4.21±0.28 (56)	3.1–5.4
Postmenopausal females*	3.74±2.43 (65)	0.63–10.03	0.81±0.47 (65)	0.25–2.22

Number of samples indicated in parentheses.
* ±SD.

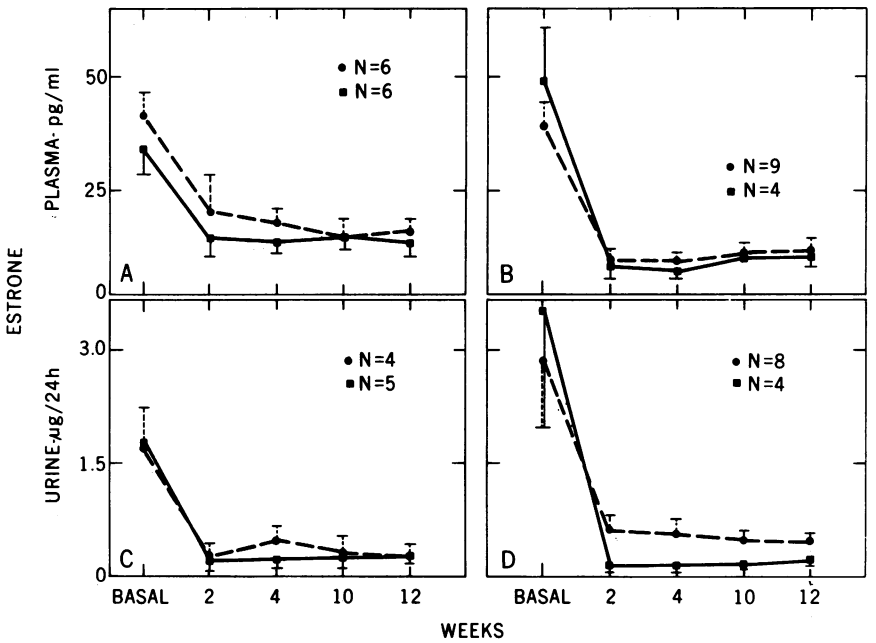


FIGURE 1 Plasma estrone levels in women treated with AG (panel A) or with surgical adrenalectomy (panel B). Urinary estrone levels in women treated with AG (panel C) or with surgical adrenalectomy (panel D). Data are mean±SEM for subgroups of women who are in spontaneous menopause (●) or postoophorectomy (■). N represents the number of patients. No statistical significance between subgroups was detected.

postmenopausal ovary to the hormone levels measured during therapy, data from surgically castrate (oophorectomy) and spontaneously menopausal women were examined separately (Figs. 1 and 2A–D). No consistent differences were observed between groups during the basal observation period as shown in Figs. 1 and 2B and D. After surgical adrenalectomy, the mean plasma and urinary E_1 and E_2 levels

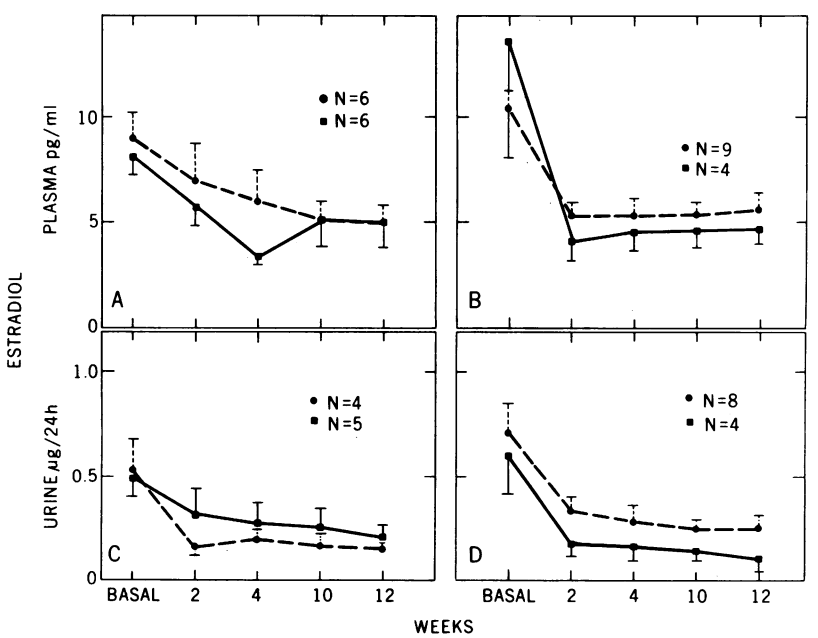
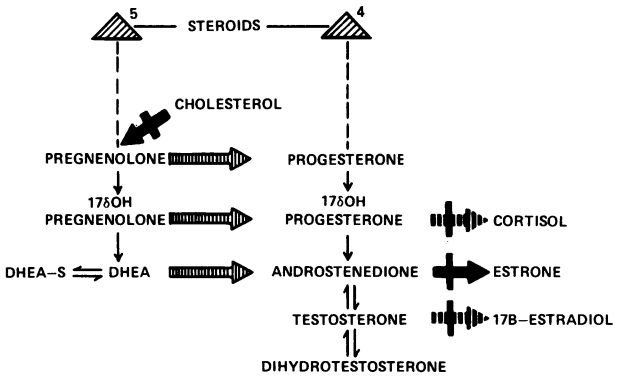


FIGURE 2 Plasma and urinary estrogens are depicted exactly as in Fig. 1.

were slightly higher in spontaneously menopausal women compared with castrate (oophorectomy) women over 16/16 observation periods (i.e., week 2, 4, 10, and 12 for plasma E_1 and E_2 and urine E_1 and E_2, Figs. 1 and 2B and D). In contrast, during AG treatment, the levels of these estrogens in surgically castrate women were not consistently lower than in spontaneously menopausal women (5/16 instances lower, 6/16 higher; Figs. 1 and 2A and C). The two groups did not differ significantly by analysis of variance or covariance of the group trends, or by *t* test analysis of individual time points.[2] For DHEA-S and Δ^4-A, the mean basal and treatment concentrations compared between the spontaneously menopausal and surgically castrate groups did not differ significantly at any time point. Similarly, no significant differences between the two groups were observed for T, DHT, LH, FSH, or prolactin (data not shown).



POTENTIAL SITES OF ACTION OF AG ON THE INHIBITION OF SYNTHESIS OF PREGNENOLONE ( ), CORTISOL( ), ESTRONE ( ) AND ESTRADIOL ( ). PRESERVATION OF TESTOSTERONE AND DIHYDROTESTOSTERONE SECRETION IS INDICATED BY SMALL ARROWS ( ). THE LARGE STRIPED ARROWS ( ) REPRESENT THE 3β-oL-DEHYDROGENASE, Δ^5 TO Δ^4-ISOMERASE ENZYME SYSTEM WHICH MAY BE ENHANCED IN THE DIRECTION SHOWN DURING AG TREATMENT.

FIGURE 3 See legend appended to figure.

DISCUSSION

AG is an inhibitor of steroid biosynthesis; it binds to the cytochrome P-450 complex to block several steroid hydroxylation steps (32). Initial studies demonstrated that AG inhibits the conversion of cholesterol to pregnenolone by interfering with 20α-hydroxylation (17). Subsequent investigations revealed inhibitory effects on the three 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 (19). In such patients, a similar inhibition of androgen production would be predicted if AG predominantly blocked cholesterol to pregnenolone conversion, a step required for androgen biosynthesis. However, the present study demonstrated preservation of T and DHT plasma levels during estrogen suppression in women receiving AG.

Analysis of the known actions of AG (Fig. 3) suggests a possible mechanism for the observed preservation of androgen secretion. AG, in the doses employed in this study (1,000 mg daily), only partially inhibits the 20α-hydroxylation of cholesterol. As a reflection of incomplete blockade, certain steroids requiring this step for synthesis, such as 17α-hydroxypregnenolone, DHEA, and DHEA-S, were still measurable during drug treatment, albeit at markedly reduced levels (23).

Previous studies suggested that AG induces an alteration in the intraadrenal conversion of the Δ^5- to Δ^4-steroids through an effect on the 3β-ol dehydrogenase, Δ^5- to Δ^4-isomerase enzyme complex (23). This action of AG results in preferential conversion of the Δ^5-steroid precursors to progesterone, 17α-hydroxyprogesterone, 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 (33). Consequently, early steroid precursors that escape the blockade of 20α-hydroxylation are preferentially shunted into the androgen pathway and are secreted as Δ^4-A, T, and DHT. The observed elevations of progesterone, 17α-hydroxyprogesterone, and Δ^4-A during AG in our previous studies are also explained by this hypothesis (18, 23). Finally, the secretion of these steroids is further increased if glucocorticoid replacement is not given concomitantly with AG to prevent reflex ACTH increments. This mechanism produced the strikingly elevated levels of Δ^4-A reported by Newsome et al. (15) and could explain the earlier report of hirsutism occurring in women receiving AG without hydrocortisone supplementation (34).

Other actions of AG could also explain the preservation of plasma androgen levels concomitant with the marked suppression of cortisol, 17α-hydroxypregnenolone, DHEA, DHEA-S, and the estrogens that were observed in our previous and present studies (Tables I, II) (23, 35). A preferential reduction in the rate of androgen metabolism, for example, could produce the steroid pattern observed. One might predict such an effect because AG inhibits the metabolism of androgens to estrogens (19). Further, Horky et al. (36) suggested that AG might alter the pattern of peripheral metabolism of T in castrate men. However, we have excluded this possibility with respect to one

[2] Nonparametric tests of statistical significance were not performed because of the dependent nature of the individual values between time points and between assays in the same patients.

androgen, Δ^4-A, by demonstrating that its metabolic clearance rate is not influenced by AG (19). A decrease in the metabolic clearance rates of T and DHT is considered unlikely. AG accelerates the metabolism of certain steroids (e.g., dexamethasone), does not alter that of others (e.g., cortisol, Δ^4-A, E_1, and medroxyprogesterone acetate), but has not been shown to reduce the rate of steroid degradation (19, 35, 37, 38). Consequently, the most likely explanation for maintenance of androgen concentrations during AG therapy is increased conversion of Δ^5- to Δ^4-steroids in concert with C-11 and C-21 hydroxylase inhibition. However, direct isotopic kinetic studies are necessary to prove this hypothesis conclusively.

The suppression of estrogen production with preservation of androgen levels in women with metastatic breast carcinoma might produce beneficial effects on tumor growth. For years androgens have been used in the treatment of metastatic or inoperable breast carcinoma (39, 40). In women with metastatic breast cancer who are fewer than 5-yr postmenopausal and whose tumors are estrogen-receptor positive, remissions may be induced by androgens with greater frequency than by estrogens (41). Women with metastatic breast carcinoma treated with antiestrogens in combination with androgens appear to experience tumor regression more frequently than patients treated with antiestrogens alone (42). The mechanism by which androgens exert their effects on breast carcinomas and the amount of androgen required (i.e., physiologic vs. pharmacologic amounts) for tumor regression have not been fully clarified.

Most likely, inhibitory effects of androgens on tumor growth could occur by the reduction of peripheral aromatization of androgens to estrogens. These compounds appear to inhibit the process by binding to placental microsomal cytochrome P-450, the enzyme system that is essential for placental aromatization of Δ^4-A (43–45). It is possible that similar mechanisms may be involved with the inhibition of peripheral aromatization. The finding that some androgens inhibited placental aromatization suggested that the underlying mechanism of androgen therapy of human breast cancer may be in the reduction of endogenous estrogen production by inhibition of peripheral aromatization rather than in a direct androgenic effect of these compounds on the tumor cells. However, androgens can exert their effect on aromatization in vitro (46) and can directly inhibit the cellular growth of breast tumor cells in culture (47). In a number of clinical circumstances, as pointed out by Siiteri et al. (46), an alteration of the ratio of androgen to estrogen circulating in plasma can influence the responsiveness of normal breast tissue. Finally, Bulbrook et al. (48–50), Poortman (51), Poortman et al. (52), and Thijssen (53) correlated the pattern of androgen production and excretion of urinary metabolites with responsiveness to endocrine therapy in women with breast carcinoma. Their observations also suggest a possible role for androgens in the growth of breast carcinoma.

It remains to be demonstrated, however, that the preservation of androgen secretion in our patients is of biologic significance. Further follow-up of the patients treated with surgical adrenalectomy and with AG should provide additional insight into this possibility.

Other agents used in therapy of metastatic breast carcinoma also inhibit the biologic effects of estrogens preferentially. The antiestrogen, tamoxifen, antagonizes estrogen action but has not been shown to block androgen activity or secretion in postmenopausal patients (54). Other aromatase inhibitors such as testolactone (Teslac, E. R. Squibb & Sons, Princeton, N. J.) or Δ^4-androstenetriene also inhibit estrogen production without altering androgen secretion in women or in rats with mammary carcinoma (55). Teslac, however, lowers only E_1 and not E_2 levels, and, consequently, modifies androgen:estrogen ratios less than AG (56). At the present time, insufficient clinical data with aromatase inhibitors or antiestrogens have been accumulated to assess the importance of continued androgen secretion in such estrogen-deprived patients with metastatic breast carcinoma.

Numerous studies have demonstrated that breast neoplastic cells are highly sensitive to the amounts of estrogen produced basally in postmenopausal women. Tumor regressions commonly occur in response to the reduction in estrogen induced by surgical adrenalectomy. It was of interest in this study to investigate whether other tissues, such as the pituitary and hypothalamus, are equally sensitive to small estrogen fluxes. Surprisingly, surgical adrenalectomy, although lowering E_1 and E_2 levels significantly, did not induce a rise in serum LH or FSH. This finding was confirmed by a similar lack of gonadotropin increments in women receiving AG. These data suggest that the hypothalamic-pituitary axis in postmenopausal women is not sufficiently sensitive to respond to the negative feedback effects of adrenally related estrogens. The alternate explanation, that the pituitary secretes gonadotropins maximally under these circumstances, is unlikely since exogenous gonadotropin-releasing hormone can stimulate marked increases in LH and FSH secretion in postmenopausal women (57).

Prolactin levels were measured in this study to assess the effects of surgical adrenalectomy on secretion of this hormone. Sarfaty et al. (58) previously reported that surgical adrenalectomy may induce chronic increments in prolactin secretion. Such an elevation of prolactin could potentially result in more rapid tumor growth in women with breast carcinoma. However, we were unable to confirm Sarfaty's (58) observation and found no significant differences in prolactin levels

after surgical adrenalectomy or after AG (Table III; 59). It may be that the stress of surgery and later continued tumor burden may explain the variability of prolactin levels measured in individual patients and the discrepancy between our results and those of Sarfaty (58).

One important goal of this study was to determine whether plasma and urinary estrogen levels are lowered by AG to the levels produced by surgical adrenalectomy. In response to either therapy, E_1 fell by 62–75% in plasma and 85–88% in urine (Table I) and E_2 by 40–72% in both biologic fluids. Among 16 intergroup comparisons between the two treatment modalities (i.e., at week 2, 4, 10, and 12 for plasma and urine E_1 and E_2), only one was significantly lower in the women treated surgically (wk 4, plasma E_1) (Figs. 1 and 2). Similar estrogen suppression also occurred in subgroups of spontaneously menopausal and surgically castrate women. Although both estrogens generally remained higher in spontaneously menopausal than castrate women during either treatment, no significant differences between these two subgroups could be demonstrated. From these data, it is possible to conclude that AG treatment produces estrogen suppression equivalent to that induced by surgical adrenalectomy.

The side effects of AG in patients treated with the regimen described are of interest. As reported previously (60), lethargy, skin rash, ataxia, and drug fever frequently occur during the first 6 wk of therapy but resolve spontaneously with chronic therapy. Because of these side effects, cessation of treatment is required in only 10% of the patients.

In conclusion, equivalent and highly significant estrogen suppression occurs with either AG or surgical adrenalectomy. Marked dissociation of the extent of androgen inhibition was observed between these two groups of patients. AG permitted relative preservation of androgen secretion compared with surgical adrenalectomy. Further studies are necessary to evaluate the clinical significance of these findings in influencing breast carcinoma growth and regression.

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