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CLINICAL USE OF AROMATASE INHIBITORS: CURRENT DATA AND FUTURE PERSPECTIVES

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INTRODUCTION

The aromatase enzyme catalyzes the rate-limiting step in estrogen biosynthesis, the conversion of androstenedione to estrone.[1] Three separate steroid hydroxylations are involved in this step. Each utilizes NADPH as cofactor and an aromatase-specific cytochrome P-450 to insert molecular oxygen into the steroid structure. Sequentially, two of these hydroxylations take place at the C_{19} carbon position. The site of the final step is controversial and not fully established. After the three hydroxylations are completed, a non-enzymatic condensation takes place which releases formic acid and results in the formation of estrone.[2,3]

In premenopausal women, the most important site of aromatase is the ovary. Regulation of ovarian estradiol synthesis is complex and involves two cellular compartments. In the granulosa cell compartment, FSH stimulates the activity of aromotase by increasing the amount of aromatase messenger RNA and the number of molecules of enzyme. In the theca cell compartment, the stubstrate of aromatase, androstenedione, is stimulated by LH.[4]

Because of the complexity of regulation in the ovary, pharmacologic blockade of ovarian aromatase has been difficult in patients. Interruption of estrogen biosynthesis reduces the tonic inhibitory action of estradiol on LH and FSH secretion. The reflex rise in FSH stimulates production of new aromatase enzyme. The LH increment results in enhanced ovarian steroidogenesis in the thecal compartment, and specifically in higher amounts of the aromatase substrate, androstenedione. These two effects tend to counteract the inhibitory actions of non-steroidal aromatase blocking drugs on the ovary.

In postmenopausal women, the granulosa cell compartment of the ovary is lost and aromatase activity is markedly reduced. Estrogen synthesis takes place nearly exclusively in extraglandular tissues in such individuals.[6] Extraglandular aromatase is present predominantly in fat, liver, muscle, and hair follicles.[7] Androstenedione serves as the predominant substrate for aromatase, while testosterone represents only a minor precursor. The adrenal directly secretes androstenedione which enters tissue for aromatization to estrone. The enzyme, 17-beta-hydroxysteroid dehydrogenase, then converts estrone to estradiol. A small fraction of androstenedione is secreted by the ovaries as well.[8] For that reason, precise measurement of estrogens reveals slightly lower values in surgically oophorectomized than in spontaneoulsy menopausal women.[9]

Through the androstenedione to estrone pathway, postmenopausal women produce approximately 100 μg of estrone/day and more, if obese.[10] A substantial fraction of estrone is converted to estradiol to produce circulating concentrations of 10–20 pg/ml. This level of estradiol should not be sufficient to occupy a biologically meaningful fraction of tumor estradiol receptors.[11] However, the levels of estradiol in tumor tissue are an order of magnitude higher than in plasma and, thus may be sufficient for an important level of receptor occupancy.[12,13] The mechanisms responsible for maintenance of high tissue estradiol concentrations are not clearly defined at present but could potentially involve local production via either aromatase or sulfatase[14] in the tumor or in tissue surrounding the tumor. Several investigative groups identified aromatase activity in human breast tumors.[15–17] Compared to human placenta, absolute levels of activity are relatively low, ranging from 5–80 pmol/gprotein/hr. Bradlow regarded this degree of activty to be too low for a meaningful level of estradiol to be synthesized locally.[11] Aromatase, however, could be localized to specific cell types such as adipose cells, stroma or certain epithelial tumor cells.[15] If correct, biochemical measurement of total aromatase activity would underestimate the levels of enzyme activity present, for example, in isolated epithelial tumor cells.

The biologic importance of *tumor* aromatase rests in the concept that aromatase inhibitors would block estradiol synthesis directly at the site of the tumor. Indirect support for this hypothesis comes from preliminary studies correlating tumor aromotase activity with clinical responses to aromatase inhibition.[18,19] Prospective trials of responses to aromatase inhibition in aromatase-rich and aromatase-poor tumors are now required to critically examine this tissue. The author of this review favors the argument of Bradlow that tumor aromatase is too low for biologic significance.[11] Local estrogen production is more likely to result from conversion of estone sulfate to estrone via the enzyme, sulfatase.[14] Nonetheless, production of estrone sulfate in *peripheral tissues* still requires aromotase as the rate-limiting enzymatice step.

CLINICAL USE OF THE AROMATASE INHIBITOR, AMINOGLUTETHIMIDE

Endocrine Effects

Aminoglutethimide was initially recognized to be an inhibitor of cytochrome P450-mediated steroid hydroxylations and particularly of those involving the cholesterol side-chain cleavage enzyme.[20] The first clinical use of aminoglutethimide for breast cancer attempted to produce a "medical adrenalectomy" by blocking cholesterol side-chain cleavage.[21,22] Replacement glucocorticoid was added to compensate for the inhibition of cortisol biosynthesis. While these studies were ongiong, however, Siiteri *et al.* brought attention to the potent aromatase inhibitory properties of aminoglutethimide *in vitro*.[23,24] Since each of the 3 hydroxylations catalyzed by aromatase requires the participation of a cytochrome P450 step, it is not surprising that aminoglutethimide blocks aromatase. Direct isotopic kinetic studies in patients then confirmed the potency of aminoglutethimide as an aromatase inhibitor *in vivo*.[25] A dose of 1000 mg of aminoglutethimide daily produced 95-98% inhibition of aromatase in postmenopausal women with breast cancer.

The regimen of aminoglutethimide plus hydrocortisone inhibited plasma and urinary estradiol to levels comparable to those observed following surgical adrenal-

ectomy[26] or hypophysectomy in patients with breast cancer. However, as Siiteri had suggested, the primary effect of this drug in lowering estrogen production was inibition of the aromatase enzyme. This conclusion was inferred from the observation that androstenedione levels were unchanged (or even increased), whereas estrogen levels fell profoundly during the administration of aminoglutethimide and hydrocortisone.[27] The lack of a decrease in androstenedione was initially unexpected. However, this preservation of androgen secretion, which included testosterone and dihydrotestosterone as well as androstenedione, was later explained by the blocking effect of aminoglutethimide on another P_{450}-mediated step, the 11 β-hydroxylase enzyme.[28] Enhanced conversion of delta-5 to delta-4 steroids was also reported to occur.[29]

Comprehensive studies of the endocrine effects of aminoglutethimide plus hydrocortisone identified several additional actions (Table I). These included blockade of aldosterone and thyroxine synthesis as well as enhancement of estrone sulfate metabolic clearance rate.[30,31] The latter effect has been proposed as an additional mechanism for lowering of plasma estrogen levels. Estrone sulfate can be converted to estrone through the enzyme, sulfatase. Lowering of estrone sulfate levels through enhancement of clearance should result in a reduction in the amount of substrate available for sulfatase, and thus the amount of estrone produced though this mechanism. Aminoglutehimide also mediates several other important metabolic alterations (Table I).

Clinical Effects

A compilation of clinical responses to aminoglutethimide plus glucocorticoid in women with breast cancer reveals results similar to those expected with other forms of endocrine therapy (Figure 1).[32] Overall, one-third of women experience either complete or partial tumor regression whereas 54% with estrogen receptor positive tumors respond with objective regressions.[32] Responses persist for a mean of 13 months and patients survive for an average of 20 months. By site of disease, soft tissues respond most frequently followed by lymph nodes, bone, lung/pleura, viscera, and liver. These results are similar to those observed in patients receiving tamoxifen, medroxyprogesterone acetate, or megestrol acetate under similar circumstances (Figure 1).[32]

Side Effects

Patients receiving standard dose aminoglutethimide experience a wide range of side effects during the induction of therapy (Table II). The major problems encountered include drug rash, fever, and lethargy. These precluded continuing treatment in 8–15% of patients and particularly in elderly women. Many of these symptoms resolve completely or diminish in severity with treatment for longer than 6 weeks. One physiologic basis for reduction in side effects is the fact that aminoglutethimide accelerates its own metabolism from 12 h to approximately 7 h presumably through hepatic enzyme induction.[33] Skin rash is a particular problem but resolution occurs spontaneously in the majority of patients without discontinuation of therapy. Approximately one-third of women require mineralocorticoid replacement with florinef® because of the inhibition of aldosterone production.[26] Another 5% of patients require thyroxine supplementation because of the effects of aminoglutethimide

TABLE I
Endocrine and Metabolic Effects of Aminoglutethimide

Metabolic Step	Action	Measurable Results	Clinical Import	Clinical Implications	Ref
Aromatase enzyme	inhibition	reduction estrogen synthesis	major	mechanism of action -breast tumor regression	(23,24,25)
Estrone sulfate metabolic clearance	acceleration	reduction plasma estrone levels	uncertain	additional mechanism -breast tumor regression	(31)
CSCC	inhibition	reduction of cortisol biosynthesis	major	requirement for glucocorticoid replacement although effect usually overcome by reflex ↑ in ACTH (with AG, 1000 mg alone)	(74)
11-OHase	inhibition	↑ 17α-OH-prog, Δ_4-A, T, DHT in absence of exogenous glucocorticoid administration	virilization can occur when AG given without exogenous gluco-corticoid	explains lack of Δ_4A suppression with combination AG + Glucocorticoid	(28)
C-21-OHase	inhibition	↑ 17α-OH prog levels	minor	none	(74,75)
C-18-OHase	inhibition	partial reduction in aldosterone levels	major	one-third of patients require mineralocorticoid replacement	(76)
3β-ol dehydrogenase	enhanced	↑ ratios			(29)
5→ 4 isomerase	$\Delta_5\Delta_4$ isomerase conversion	Δ4/5 steroidal pairs	minor	none	
hepatic 6β-hydroxylase	enhanced 6β hydroxylation synthetic gluco -corticoids	accelerated metabolic clearance rate-dexamethasone	enhanced requirement for synthetic glucocorticoid	3 mg dexametheasone needed as glucocorticoid replacement in patients receiving aminoglutethimide	(77)
thyroxine biosynthesis	inhibition	lowered thyroxine, ↑ TSH levls	5% of pts develop hypothyroidism	must monitor thyroid function in patients on AG	(78)

coumarin metabolism	acceleration	prothrombin time lowered in pts receiving coumarin	drug-drug interaction	coumarin dose adjustment to compensate	(79)
prostaglandin biosynthesis	inhibition	*in vitro* only	uncertain	may partially explain relief of bone pain in patients with breast cancer metastases	(80)
2-hydroxylase	inhibition	*in vitro* only	uncertain	uncertain	(81)
AG metabolism	accelerated	reduces AG blood levels	major	gradual dose escalation advised	(33)

- CSCC = cholesterol side-chain cleavage
- 11-OHase = 11-hydroxylase
- C_{21}-OHase = 21-hydroxylase
- C_{18}-OHase = 18-hydroxylase
- Δ_4-A = androstenedione
- T = testosterone
- DHT = dihydrotestosterone
- AG = aminoglutethimide

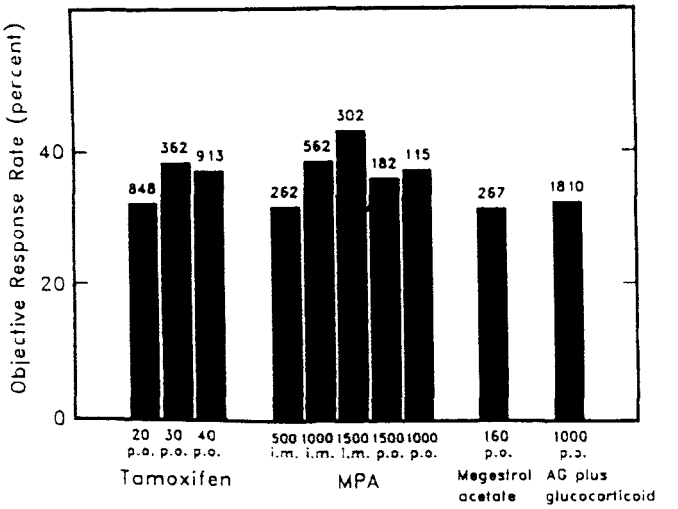

FIGURE 1 Comparative rates of objective responses (i.e., complete and partial objective regression) to various endocrine therapies. Data are from non-randomized trials and represent patients receiving first-line or subsequent endocrine therapy. The numbers above the bars represent the number of patients in each category. Adapted from Petru *et al.*[32]

TABLE II
Dose-related Side Effects and Toxicity of Aminoglutethimide

	Aminoglutethimide without Hydrocortisone			Aminoglutethimide with Hydrocortisone		
	250 mg	500 mg	1000 mg	250 mg	500 mg	1000 mg
Signs/Symptoms (%)						
Rash	15 16	12	23	22	4 3	23 11
Drowsiness, fatigue, lethargy	31 0	40	62	9	9 23	33 17
Nausea	12 4	4	26	9	4 3	15 8
Ataxia	0 0	0	0	4	3 0	4 10
Drugs Discontinued (%)	8 1	0	11	1	5 0	5 7
Number of Patients	65 57	28	47	101	78 29	213 83
Study Reference	(36) (39)	(45)	(82)	(41)	(83) (45)	(83) (84)

to inhibit thyroid hormone synthesis.[34] In the remainder, TSH levels increase sufficiently to completely overcome the blockade of thyroxine biosynthesis. The frequency and severity of side effects, particularly when compared to tamoxifen administration, has led to attempts to reduce aminoglutethimide dosage and to develop second and third generation aromatase inhibitors.

LOW DOSE AMINOGLUTETHIMIDE WITH HYDROCORTISONE

In seeking lower doses of aminoglutethimide to reduce side effects, several investigators conducted dose-response studies using complete aromatase inhibition as the endpoint to judge sufficiency of dosage.[35–38] With this criterion, 125 mg of aminoglutethimide daily blocked aromatase significantly and 250–500 mg daily produced

maximum inhibition. Studies which were predominantly non-randomized then compared 250, 500 and 1000 mg of aminoglutethimide daily in combination with hydrocortisone (Table III). The idiosyncratic reactions such as skin rash occurred as commonly with low as with high dose therapy. Side effects such as lethargy, fatigue and drowsiness, which could be attributed to the CNS sedative propeties were still reported but somewhat less frequently with lower doses (Table II). No cases of agranulocytosis were observed in patients receiving 250 mg of aminoglutethimide daily[39–41] whereas 1–2% of patients receiving 1,000 mg of aminoglutethimide daily experience this toxicity.[42] Clinical responses to low dose aminoglutethimide plus hydrocortisone appeared equal to those observed with higher doses of aminoglutethimide (Table III).

LOW DOSE AMINOGLUTETHIMIDE WITHOUT HYDROCORTISONE

Reduction of aminoglutethimide dosage sufficiently should abrogate inhibitory effects on cholsterol side-chain cleavage and other enzymes involved in cortisol biosynthesis and eliminate need for replacement glucocorticoid. This strategy would provide "pure" aromatase inhibition and eliminate need for exogenous hydrocortisone. Two studies (Table III) utilized this strategy and employed 125 mg twice daily of aminoglutethimide without glucocorticoid.[39,40,43] Plasma estrogens fell to the same extent as had been demonstrated in prior studies with high doses of aminoglutethimide plus hydrocortisone. However, objective tumor regressions occurred in only 16–19% of patients and response rates appeared somewhat lower than expected. In addition, dose escalation either to 750 or 1000 mg of aminogluthimide with addition of glucocorticoid produced additional responses in 18–23% of patients.[39,40] In addition, Dowsett *et al.*[44] suggested that addition of hydrocortisone to 250 mg of aminoglutethimide daily produced further suppression of estrogens than did aminoglutethimide alone. They attributed this reduction of estrogens to an effect of hydrocortisone to lower the levels of androstenedione as substrate in a setting of incomplete aromatase inhibitory doses of aminoglutethimide (i.e., 250 mg). However, other studies demonstrated equal estrogen suppression with and without hydrocortisone supplementation and this issue remains controversial.

A recent study[45] compared the use of aminoglutethimide with and without hydrocortisone in a randomized, controlled trial. Five hundred mg of aminoglutethimide alone was compared with 500 mg of aminoglutethimide plus 40 mg of hydrocortisone as first-line therapy in estrogen positive or unknown patients. Estrogen levels fell similarly with either regimen. Complete and partial objective regression did not differ (i.e., 45% AG + HC, 41% AG alone) nor did duration of remission and survival. These data suggest that addition of hydrocortisone adds little to the therapeutic efficacy of the aromatase inhibitor given alone provided that the dosage of aromatase inhibitor is sufficient.

In summary, the optimal aminoglutethimide regimen is not yet clearly established. The author favors use of 250–500 mg of aminoglutethimide daily in combination with 40 mg of hydrocortisone until further randomized data are available.

TABLE III
Studies of Varying Doses of Aminoglutethimide with and without Glucocorticoid

Regimen	Number of Patients	Overall Objective Responses	Mean Duration responses (mo)	Mean Duration Survival (mo)	Ref	Comments
STUDY DESIGN — NONRANDOMIZED:						
Aminoglutethimide without *Glucocorticoid*						
AG, 250 mg	25	16%	-	-	(40)	• (*18% with objective response to dose escalation to 1000 AG, 40 mg HC)
AG, 250 mg	57	19%	8+	-	(39)	• (23% with objective response to dose escalation to 750 AG, 37.5 mg cortisol acetate)
AG, 250 mg	57	19%	-	-	(39)	
AG, 375 mg	19	26%	-	-	(40)	
AG, 500 mg	27	33%	-	-	(40)	
AG, 1000 mg	38	13%	13	-	(85)	• extensively pretreated patients
Aminoglutethimide with *Gluocorticoid* AG, 250 mg HC, 40 mg	101	25%	12 (median)	18 (median)	(41)	• 73%with prior endocrine therapy; 56% response rate in patients with no prior endocrine therapy
AG, 500 mg HC, 40 mg	76	33%	-	-	(86)	
AG, 500 mg HC, 40 mg	19	26%	-	-	(83)	• Similar response as in patients randomized to 1000 mg AG, 40 mg HC
AG, 1000 mg	1810	33%	13	20	(32)	• Compilation of data from multiple studies
Gluc, 20-50						

STUDY DESIGN: RANDOMIZED						
Aminoglutethimide with and without *Glucocorticoid*						
AG, 500 mg	73	41%	11	26	(45)	•Randomized trial • First-line therapy
vs.						
AG, 500 mg HC, 40 mg	75	45%	13	27		

DEVELOPMENT OF IMPROVED AROMATASE INHIBITORS

Background

The successful use of aminoglutethimide provided an impetus to investigate further the concept of inhibiting estrogen biosynthesis as a means of treating breast cancer. However, the problems with the multiple actions of aminoglutethimide and its associated side effects, spurred interest in the aromatase inhibitors originally reported by Brodie and colleagues. Compounds such as 4-hydroxyandrostenedione were designed as selective inhibitors of aromatase with greater potency than aminoglutethimide. A wide variety of compounds including 4-hydroxyandrostenedione are now under study (Table IV).[46] A convenient classification divides inhibitors into the mechanism based or "suicide inhibitors" and those of the competitive type. "Suicide" inhibitors initially compete with the natural substrate (i.e., androstenedione and testosterone) for binding to the active site of the enzyme. The enzyme, then, specifically acts upon the inhibitor to yield reactive alkylating species which can form covalent bonds at or near the active site of the enzyme. Through this mechanism, the enzyme is irreversibly inactivated. Competitive inhibitors, on the other hand, bind reversibly to the active sites of the enzyme and prevent product formation only as long as the inhibitor occupies the catalytic site. Whereas mechanism-based inhibitors are exclusively steroidal in type, competitive inhibitors consist both of steroidal and nonsteroidal compounds. Tables IV and V and VI provide a practical listing of aromatase inhibitors highlighting the most potent or most interesting of each series of compounds.

Mechanism-based or "suicide" inhibitors should be preferable to competitive inhibitors because of their irreversible nature. Theoretically, their duration of action *in vivo* should be prolonged and related primarily to the rate at which new enzyme can be synthesized. These concepts require experimental verification.[47]

Specificity of inhibition as well as intrinsic biologic activity are important considerations regarding aromatase inhibitors.[46] In general, nonsteroidal inhibitors are more likely than are sterodial compounds to lack specificity since they have a potential for blocking several cytochrome P_{450}-mediated steroid hydroxylations. On the other hand, steroidal inhibitors or their metabolites have greater potential for producing estrogen, androgen, glucocorticoid or progestin agonist or antagonistis effects through the inherent properties of their structures. The aromatase inhibitors presently in clinical trials are indicated in Table V and VI.

Mechanism-Based ("Suicide") Inhibitors

PED This steroidal derivative is both a potent competitive inhibitor of aromatase as well as a mechanism-based agent with an inactivation constant[46] (K) inact of $1.1 \times 10^{-3} S^{-1}$. *In vivo*, this compound causes regression of DMBA-induced rat mammary tumors and blocks aromatase activity in JAR (human choriocarcinoma) cell growth in nude mice.[48,49] Its duration of action in monkeys[50] suggests that enzyme inactivation may play a biologically meaningful role in its mode of blocking aromatase. Initial studies in patients are just beginning and no published data are available.

4-Hydroxyandrostenedione This compound competitively inhibits aromatase *in vitro* and exhibits mechanism-based inactivation with a K_{inact} of $4.1 \times 10^{-3} S^{-1}$. 4-Hydroxyandrostenedione effectively blocks estradiol prodution *in vivo*. Extensive studies

TABLE IV
Partial List of Aromatase Inhibitors

Type of Inhibition	Type of Compound	Name of Compound	K_i	K_{inact}	Ref
Mechanism-Based	steroid	1,4,6-androst-triene-3,17-dione		$1.1 \times 10^{-3} S^{-1}$	(46)
	steroid	4-OH-androstene-dione		$4.1 \times 10^{-3} S^{-1}$	(87)
	steroid	4-androstene-3,6, 17-trione		$4.03 \times 10^{-3} S^{-1}$	(87)
	steroid	testolactone		$5.5 \times 10^{-4} S^{-1}$	(46,87)
	steroid	10β-propargylestr-4-ene-3,17-dione		$1.11 \times 10^{-3} S^{-1}$	(46)
	steroid	7α (4′-amino) phenyl-thio,1,4-androsta-diene-3,17-dione		$8.4 \times 10^{-3} S^{-1}$	(46,88)
Competitive	steroid	1-methyl-androsta-1,4-diene,17-dione	80 nM		(46)
	steroid	6α-bromo-androstene-dione	3.4 nM		(46)
	steroid	7α(4′-amino) phenyl thio-4-androstene-3,17-dione	18 nM		(46)
	non-steroid	aminoglutethimide	540 nM		(66)
	non-steroid	pyridoglutethimide	1100 nM		(46)
	imidazole	CGS 16949A	0.19 nM		(66)
	imidazole	R-76713	0.70 nM		(65)
	imidazole	econazole	0.06 μM*		(46)

*IC50

TABLE V
New Aromatase Inhibitors Reaching Clinical Trial

Name of Compound	Type of Inhibitor	Potency for Aromatase		Inhibition of Other Enzymes				
		K_i	K_{inact}	CSCC	C17-20 lyase	C-11 hydroxylase	C-21 hydroxylase	Aldosterone inhibition
1-methyl-1,4-androstadiene-3,17-dione	competitive	80 nM	–	–	–	–	–	–
4-OH-androstenedione	mechanism-based		$4.1 \times 10^{-3} S^{-1}$	–	–	–	–	–
10β-propargyl-estr-4-ene-3,17-dione	mechanism-based		$1.11 \times 10^{-3} S^{-1}$	–	–	–	–	–
Pyridoglutethimide	competitive	100 nM	–	–	?	?	?	?
CGS-16949A	competitive	0.19 nM	–	–	–	+	+	+
R-76713	competitive	0.70 nM	–	–	±	±	–	–

TABLE VI
New Aromatase Inhibitors Reaching Clinical Trial

Name of Compound	Steroid Agonist or Antagonist Properties	Phase I Trials	Phase II Trials	Phase III Trials	Major Sides Effects	Comments	Ref
1-methyl-1,4-androstadiene-3,17-dione	unknown	+	–	–	–	lowers estrogen levels in male volunteers	(46)
4-OH-androstene-dione	A*	+	+	–	–	active in producing tumor regression (see Table VII)	(87)
10propargyl-estr-4-ene-3,17-dione	–	+	–	–	–	human data unpublished	(46)
pyridogluteth-imide	–	+	–	–	–	clinical data preliminary	(46)
CGS 16949A	–	+	+	+	–	active in producing tumore regression	(66)
R-76713	–	+	–	–	–	studies in monkeys demonstrate blockade of aromatase with isotopic methods	(65)

*A = androgenic

revealed no estrogenic, antiestrogenic, or antiandrogenic properties.[46,51-53] However, transformation to 4-hydroxytestosterone occurs and androgenic effects can be demonstrated under certain circumstances.[54] This action is biologically important under circumstances where LH and FSH feedback loops are intact. As an example, 4-hydroxyandrostenedione markedly lowers ovarian estradiol production during the estrous cycle in rats but does not result in reflex increments in LH and FSH as would be expected. Brodie *et al.* provided evidence[53] than an androgenic effect of 4 hydroxyandrostenedione was responsible for the inhibitory effect on gonadotropin secretion.

Initial studies demonstrated that 4-hydroxyandrostenedione caused tumor regression in DMBA-induced rat mammory tumors.[55] Subsequently, this agent has been studied extensively in postmenopausal women with breast cancer. In the initial endocrine study, postmenopausal women received 500–1000 mg of 4-hydroxyandrostenedione by weekly intramuscular injection.[56] Although the drug has a rapid plasma half-life, concentrations of drug during chronic therapy and one week after the last injection, ranged from 0.7–23.2 (mean = 7.8 ± 1.1) ng/ml. This presumably resulted from a depot of drug forming at the site of injection. During therapy, plasma estradiol levels fell from 7.2 ± 0.8 (SEM) pg/ml to 2.6–2.8 pg/ml from 1 to > 4 + months after initiating treatment. Although no lowering of plasma estrone was observed in this initial study, subsequent analysis by the investigators using gas chromatography/mass spectrometry did demonstrate a decrease in plasma estrone.[57]

Preliminary clinical data with 4-hydroxyandrostenedione (Table VII) demonstrated a 33% objective regression rate of breast cancer in postmenopausal patients previously treated with multiple endocrine therapies. An insufficient number of patients were evaluable to determine the predictive nature of estrogen receptor status or the sites more favorable to response to 4-hydroxyandrostenedione. Toxicity included 6 patients with sterile abscesses due to intramuscular injections, 2 of sufficient severity to warrant discontinuation of therapy. No androgenic effects were observed and particularly, no regression of LH or FSH over basal postmenopausal values.

4-Hydroxyandrostenedione has also been given orally. Even though there is a marked first-pass effect with conversion in the liver to a glucuronidated derivative, oral doses of 250 mg reduce[58] plasma estradiol by 53% and doses up to 1000 mg produce no further suppression. The response rate after 3 months of therapy was 33% for the oral route. The only serious side effect from the oral dosage was leukopenia in a single patient.[59]

Hoffken *et al.* recently conducted a large trial of 4-hydroxyandrostenedione administration in postmenopausal women.[60] Patients initially received 500 mg intramuscularly every 2 weeks for 6 weeks and then 250 mg every 2 weeks thereafter. Plasma estradiol levels fell from baseline values of 10–11 pg/ml to levels of approximatley 4 pg/ml for up to 7 months of therapy. The drug appeared specific since no reduction of cortisol or symptoms of cortisol deficiency were observed. Of 86 evaluable patients, there were 2 complete and 19 partial remissions (21/86 = 24%) and 26 with disease stabilization (30%). Side effects included minor systemic symptoms in 11% (hot flashes, constipation, alopecia and pruritus in 2 patients each, and local symptoms in 8% (3 pruritus, 1 local pain, and 1 erythema). These resulted in discontinuation of therapy in only 2% of patients. Phase III trials are now ongoing to compare this inhibitor with standard endocrine therapies.

TABLE VII
Non-Randomized Studies of 4-hydroxyandrostenedione in Postmenopausal Women with Breast Carcinoma

Dose	Number of Patients	Overall Objective Response	Mean Duration Response	Mean Duration Survival	Comments	Ref
500 mg I.M. weekly	52	33%	–	–	•dose escalated to 1000 mg weekly in 11 non-responders; sterile abscesses in 6 patients	(57)
500 mg I.M. every other week	86	24%	13+*	–	•dose reduced to 250 I.M. every other week after 6 weeks	(60)
					•no significant toxicity and side effects minimal	(57)
500 mg p.o.	24	33%	–	–		(57)

*mean not yet reached; (+) equals that mean > 13 mo

Competitive Inhibitors

Steroidal A variety of steroidal inhibitors of aromatase are currently under investigation (Table V). At present, only 1-methyl-1, 4-androstadiene-3, 17-dione has been studied in patients. Single dose administration reveals a major reduction of plasma estrogens with this compound.[61]

Nonsteroidal Pyridoglutethimide Pyridoglutethimide is a compound resulting from modifications of the structure of aminoglutethimide to enhance specificity and to reduce side effects.[46,47] This agent has a K_i for aromatase (1100 nM), somewhat higher than for aminoglutethimide (600 nM), but does not inhibit cholesterol side-chain cleavage at concentrations of up to 50 μg/ml. Tests of CNS activity in animals suggest that sedative properties, so prominent with aminoglutethimide, are lacking. This agent reduces the growth of NMU-induced mammary tumors in rats. Further studies with this compound are ongoing and new, more potent, conjoiners are being developed.

Imidazole drugs The imidazole class of compounds have potent effects on a number of cytochrome P_{450}-mediated steroid hydroxylation steps. Ketoconazole, for example, blocks C_{17-20} lyase at low concentrations and aromatase at high concentrations.[62] This observation suggests that compounds can be found which exert relatively specific effects on certain P_{450} mediated steroid hydroxylations with little activity on others. This appears to be the case since CGS 16949A and R76713 are both potent competitive inhibitors of aromatase but lack significant cholesterol side-chain cleavage activity.[63-65] Specificity is not absolute since high concentrations of drug may block other P_{450} mediated steps as well. Development of CGS 16949A has progessed furthest as this agent in now in Phase III testing.

CGS 16949A (4-(5,6,7,8-tetrahydroimidazo[1,5a]-pyridin- 5yl)benzonitrile monohydrochloride This agent is a highly potent inhibitor of aromatase with K_i of 0.19 nM (vs. 600 nM for aminoglutethimide).[63,66] Cholesterol side-chain cleavage activity is minimal but C_{11} hydroxylase inhibitory effects are observed *in vitro* at high drug concentrations (i.e., 10^{-6} M). Negligible toxicity was observed in animal studies.[64]

Regression of DMBA-induced rat mammary tumors exceeded that produced by aminoglutethimide or by tamoxifen.[64] These observations led to Phase 1 studies in patients. A dose-ranging Phase 1 study examined the effects of 0.6-16 mg of CGS 16949A daily in women with metastatic breast cancer.[67] No CNS, hematologic, biochemical, or dermatologic toxicity was observed and the drug was remarkably well tolerated. Inhibition of aromatase, as evidenced by a fall in estrone and estradiol levels without a rise in androstenedione, occurred at a dose of 0.6 mg daily (Figures 2-C, 3). Maximum inhibition of estrogen levels was observed at doses between 2 and 4 mg daily. The 2-mg daily dose resulted in estrogen suppression similar to that observed previously with 1000 mg of aminoglutethimide and 40 mg of hydrocortisone daily. The effects of CGS 16949A at this dosage appeared to be relatively specific. However, when 8-16 mg daily were administered, increments in 17α-hydroxyprogesterone, androstenedione, and testosterone occurred, suggesting blockade of the C_{11} or C_{21} hydroxylase steps in addition. At the 16 mg dose, ACTH levels increased even though basal cortisol level were unchanged, suggesting an effect on cortisol biosynthesis.[67]

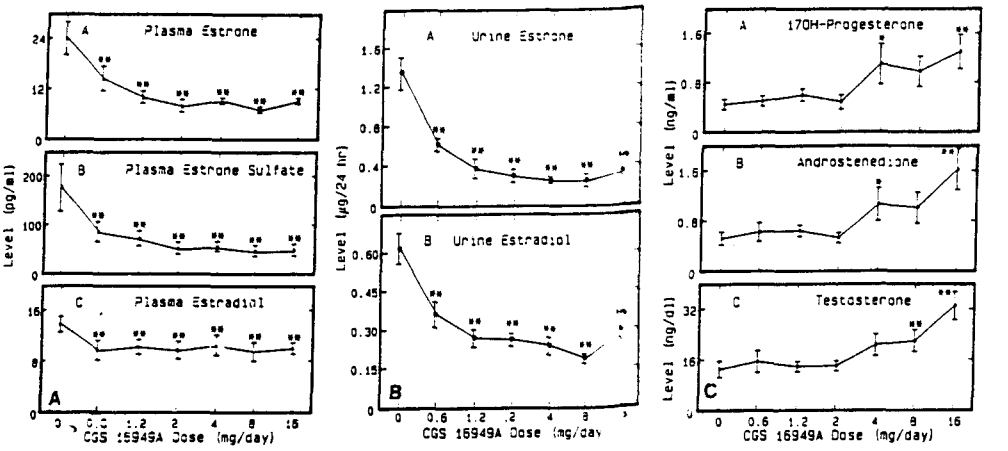

FIGURE 2 Effects of CGS 16949A on levels of plasma and urine steroids. $*p < .05$ ** $< .01$. Doses were escalated at two-weekly intervals. LEFT PANEL — plasma estrogens; MIDDLE PANEL — urine estrongens; RIGHT PANEL — plasma androgens and the precursor steroid, 17-hydroxyprogesterone. Reproduced with the permission of the *Journal of Clinical Endocrinology and Metabolism*[67].

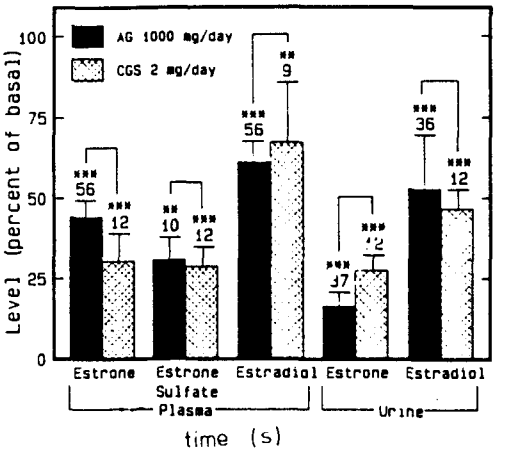

FIGURE 3 Comparison of levels of plasma and urinary estrogens in patients receiving 2 mg CGS 16949A and 1000 mg of aminoglutethimide with 40 mg hydrocortisone. Reproduced with the permission of the *Journal of Clinical Endocrinology and Metabolism*.[67]

This effect was confirmed by the demonstration of blunted cortisol responses to exogenous ACTH (cortrosyn) at this dosage. Lamberts *et al.*[68] recently demonstrated similar effects on isolated adrenal cells *in vitro* and attributed this to the C_{11}-hydroxylase inhibitory properties of the drug.

An effect on aldosterone biosynthesis was also observed at high doses since basal levels declined during the administration of 8 to 16 mg daily and ACTH stimulation of aldosterone was completely inhibited.[69] Further examination of the aldosterone pathway revealed that CGS 16949A blocks the corticosterone methyl oxidase Type II

step and increases the ratio of plasma 18-hydroxycorticosterone: aldosterone ratio as well as urinary tetrahydro-Compound A to tetrahydro-aldosterone.[69]

Results from the initial dose seeking studies led to Phase II study which compared doses of 0.6 mg 3 times daily, 1 mg twice daily, and 2 mg twice daily.[70] Maximal suppression of plasma and urinary estrogens occurred at a dosage of 1.0 mg twice daily and minimal effects on cortisol secretion were observed. Basal cortisol and ACTH levels were unaffected and cortisol levels increased to $> 20\,\mu g\%$ after exogenous cortrosyn administration in all patients. Basal levels of aldosterone also remained stable upon administration of all 3 drug dosages. No change in urinary or plasma sodium or potassium were observed, nor changes in standing blood pressure to suggest a clinical state of aldosterone deficiency. However, cortrosyn-stimulated aldosterone levels were significantly blunted at either of the 3 doses.

The antitumor activity of CGS 16949A in patients is not as yet precisely defined as limited clinical data are available. In our Phase 1 study, 2/12 heavily pretreated patients[71] experienced objective tumor regression. In the phase II trial, one complete tumor regression and 3 partial regressions were observed in the 18 patients treated in our institution.[72] Based upon similar reduction of estrogens as with aminoglutethimide and the antitumor activity of CGS 16949A in animals, it would appear that CGS would also be active in patients. The potency of the compound, its relatively specific effects on aromatase, and its lack of toxicity, suggest that it may represent a major improvement over aminoglutethimide for treatment of patients with breast cancer.

R76713 This agent represents another highly potent and specific aromatase inhibitor with little toxicity in animals studies. The Ki for placental aromatase is 0.8 nM and this agent is approximately 500-fold more potent than aminoglutethimide. Inhibition of other P_{450}-mediated steriod hydroxylations requires concentrations of 10^{-5} M or greater *in vitro*. Thus, this agent has the potential of nearly complete specificity in patients. Phase 1 clinical studies revealed an acute reduction of plasma estradiol to undetectable levels in normal men receiving one 10-mg dose and a 64% reduction in premenopausal women.[73] Clinical trials in patients with breast cancer have not, as yet, been reported.

PERSPECTIVES

Availability of newer aromatase inhibitors with high potency and specificity and lack of side effects should allow wider use of these agents for patients with breast cancer. Application in women with benign disorders such as dysfunctional uterine bleeding and leiomyomata uteri requires blockade of ovarian as well as extraglandular aromatase. The challenge will be to develop sufficiently potent inhibitors to block ovarian estrogen biosynthesis even in the face of reflex gonadotropin increments. A wide range of estrogen-dependent disorders might then be amenable to treatment with aromatase inhibitors.

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