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RECENT PROGRESS IN DEVELOPMENT OF AROMATASE INHIBITORS

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Summary—In postmenopausal women with breast cancer, aromatase, which is the enzyme converting androstenedione to estrone and testosterone to estradiol, is the rate-limiting step in estrogen biosynthesis. The currently available aromatase inhibitor, aminoglutethimide, effectively blocks estrogen production and produces tumor regressions in patients previously treated with tamoxifen. This drug, however, produces frequent side effects and blocks steroidogenic steps other than the aromatase enzyme. Thus, newer aromatase inhibitors with greater potency and specificity are under intense study. More than 20 such compounds have recently been developed. In several clinical trials, 4-hydroxyandrostenedione, given parenterally, has been highly active and specific for aromatase inhibition in patients with breast cancer. In two large recent studies, one-third of heavily pretreated women experienced objective tumor regression with this therapy. CGS 16949A, a newer agent, is also in Phase III clinical trials. This compound is an imidazole derivative with nearly 1000-fold greater potency than aminoglutethimide. An initial Phase I study compared the potency of 0.6–16 mg daily in 12 postmenopausal women and found maximal suppression of urinary and plasma estrogens with 2 mg daily. The degree of inhibition was similar to that induced by aminoglutethimide or by surgical adrenalectomy. No CNS, hematologic or biochemical toxicity was observed. A larger Phase II study in 54 patients confirmed this high degree of potency of CGS since a plateau effect was observed at the 1.8, 2 and 4 mg daily doses. The endocrine effects were not absolutely specific as a blunting of ACTH-stimulated but not basal aldosterone levels were observed. This and other emerging aromatase inhibitors offer promise as pharmacologic methods to inhibit estrogen production specifically and without side effects.

INTRODUCTION

Human breast carcinomas can be divided into two biologic subtypes: those which are hormone-dependent and those which are hormone-independent [1]. The predominant steroidal mitogen for hormone-dependent breast cancer is 17β-estradiol. The rate limiting step in the biosynthesis of estradiol is the enzyme aromatase [2]. Inhibition of this enzyme has been identified as a rational strategy for treatment of breast carcinoma. A number of other malignancies may also depend to some degree upon estrogen for their growth. Five to fifteen percent of carcinoid tumors, malignant melanomas, colo-rectal neoplasms, as well as prostate, ovarian and renal cell carcinomas have been reported to regress upon administration of the antiestrogen tamoxifen [3]. With development of highly specific and potent aromatase inhibitors, these compounds might be utilized in patients with this diverse group of neoplasms as well. Even though the response rates are low, agents with minimal toxicity could be given even with the expectation that only a small number of patients would respond.

Aromatase inhibitors in breast cancer

Aromatase inhibitors are potentially useful in both pre- and postmenopausal patients with breast cancer. However, prior studies indicate that the ovary is relatively resistant to the inhibitory effect of aromatase inhibitors. Blockade of estradiol biosynthesis results in reflex increments in the secretion of LH and FSH by the pituitary [4]. LH stimulates the interstitial cell compartment of the ovary to produce increasing amounts of the aromatase substrate, androstenedione. Increments in FSH stimulate the production of the enzyme aromatase [5]. In concert, these two actions tend to overcome the blocking effects of the aromatase inhibitors on the premenopausal ovary. Until highly potent agents are available, the majority of clinical studies will be directed toward postmenopausal patients.

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The source of estrogen in postmenopausal women is predominantly extraglandular tissue [2]. The adrenal secretes androstenedione which enters plasma and then extraglandular tissue. There it is convered via the enzyme aromatase to estrone which then re-enters the plasma and circulates at concentrations of approx. 20 pg/min. Within the extraglandular tissues, estrone may also be converted to estradiol through the enzyme 17β-hydroxysteroid dehydrogenase. Estradiol circulates in plasma at concentrations of approx. 10 pg/ml. Estrone, the aromatized product of androstenedione, may also be converted to estrone sulfate, an inactive estrogen which can be converted back to estrone through the enzyme sulfatase. Estrone sulfate is important because it circulates in plasma at levels one to two orders of magnitude higher than those of free estrone or estradiol [6].

A number of studies have also demonstrated production of estrogens directly in breast tumor tissues [7]. The estradiol synthesized within breast carcinoma cells would be expected to act in an autocrine fashion. Two sources of estradiol in the tumor tissue exist: that portion synthesized through the enzyme aromatase and the fraction arising from estrone sulfate converted to estrone through the enzyme sulfatase. A variety of studies have demonstrated the presence of both enzymes in human breast tumor tissue. Sulfatase, an enzyme with much lower affinity than aromatase (16 vs 0.027 μM) is present in breast tumor tissue at levels nearly six orders of magnitude higher than the levels of aromatase [8].

Two methods exist to reduce the stimulatory effects of estrogen on breast cancer growth. Antiestrogens bind to the estrogen receptor and block estrogen action. Alternatively, inhibitors of aromatase reduce the biosynthesis of estrone and estradiol and, through that mechanism, inhibit the stimulatory effects of estrogen [1]. The major practical advantage of aromatase inhibitors is that they do not exhibit complete cross-resistance with the antiestrogens in patients with breast carcinoma. Thirty to fifty percent of patients responding initially to tamoxifen and then relapsing, experience a secondary objective tumor regression when given aromatase inhibitors [1]. Recent studies by Jordan *et al.* suggest a potential mechanism for this lack of cross-resistance [9]. Human MCF-7 cell tumors, growing in nude mice, ultimately become resistant to the effects of tamoxifen. Such tumors will respond secondarily to the antiestrogen, ICI 164,384. It is known that tamoxifen has weak estrogen agonistic effects whereas ICI 164,384 is a pure antiestrogen and lacks agonist properties. It has been proposed that breast tumors can increase their sensitivity to the weak estrogen agonistic properties of tamoxifen and will thus regress upon administration of a pure antiestrogen. It has been difficult to develop pure antiestrogens with sufficient bioavailability and lack of toxicity for clinical use. One advantage of the aromatase inhibitors is that compounds can be synthesized which exhibit no hormone agonistic effects.

New aromatase inhibitors

New aromatase inhibitors are being developed which are highly-specific, active orally and have marked potency. Two general classes of aromatase inhibitors exist: suicide or mechanism-based inhibitors and competitive inhibitors. The "so-called" suicide inhibitors are acted upon specifically by the aromatase enzyme to open up high-affinity sites which then bind to the enzyme irreversibly. Competitive inhibitors can be divided into two subclasses: those which are steroidal and those which are non-steroidal. Advantages of the steroidal inhibitors are their specificity for blocking aromatase. Disadvantages are their diminished oral absorption and capability of being metabolized to sex steroid agonistic or antagonistic compounds. All of the non-steroidal inhibitors have the propensity to affect a wide range of cytochrome *P*-450-mediated steroid hydroxylations. The non-steroidal agents are readily absorbed orally and lack the capability of producing hormone agonist or antagonistic effects.

Table 1 summarizes several new aromatase inhibitors reaching clinical trial. These include two mechanism-based or "suicide" inhibitors, 4-hydroxyandrostenedione and 10β-propargyl-estr-4-ene-3,17-dione. One competitive steroidal inhibitor, 1-methyl-1,4-androstene-3,17-dione and three non-steroidal compounds, pyridoglutethimide, CGS 16949A and R76713, have been studied in patients [1]. The most potent of the competitive inhibitors appears to be CGS 16949A with a K_i of 0.17 mM. R76713 may be the most specific and lacks the ability to inhibit aldosterone or the C_{11}-hydroxylase enzyme at concentrations $< 10^{-5}$ M. However, at high concentrations (i.e. 10^{-5} M or greater), it exhibits effects on $C_{17\text{-}20}$ lyase and perhaps on the C_{11}-hydroxylase enzyme as well [1].

Table 1. New aromatase inhibitors reaching clinical trial

Name of compound	Type of inhibitor	Potency for aromatase			Inhibition of other enzymes			
		K_i	K_{inact}[a]	CSCC	C_{17-20} lyase	C_{11}-hydroxylase	C_{21}-hydroxylase	Aldosterone inhibition
1. 1-methyl-1,4-androstadiene-3,17-dione	Mechanism-based		$1.8 \times 10^{-4}\ s^{-1}$	–	–	–	–	–
2. 4-OH-androstenedione	Mechanism-based		$4.1 \times 10^{-3}\ s^{-1}$	–	–	–	–	–
3. 10β-propargyl-estr-4-ene-3,17-dione	Mechanism-based		$1.11 \times 10^{-3}\ s^{-1}$	–	–	–	–	–
4. Pyridoglutethimide	Competitive	1100 nM	–	–	?	?	?	?
5. CGS 16949A	Competitive	0.17 nM	–	–	–	+	±	+
6. R76713	Competitive	0.70 nM	–	–	±	±	–	–

Steroid agonist or antagonist properties	Phase I trials	Phase II trials	Phase III trials	Major side effects	Comments
1. Unknown	+	–	–	–	Lowers estrogen levels in male volunteers
2. A[b]	+	+	–	–	Active in producting tumor regression (see Table XV)
3. –	+	–	–	–	Human data unpublished
4. –	+	–	–	–	Clinical data preliminary
5. –	+	+	+	–	Active in producing tumor regression
6. –	+	–	–	–	Studies in monkeys demonstrate blockade of aromatase with isotopic methods

[a]Inactivation constant.
[b]A = androgenic.
Reprinted with the permission of the publisher for *Endocrine Reviews* **11** (1990) 221–265 [1].

Development of new aromatase inhibitors has generally followed a common strategy. This involved the synthesis and identification of potent and specific inhibitors; the selection of agents which produce minimal side effects and have minimal toxicity; the initiation of Phase I human studies to demonstrate estrogen suppression, determine doses, evaluate side effects and toxicity; the evolution to Phase II studies to demonstrate antitumor efficacy at optimal doses; and the initiation of large Phase III human studies to compare efficacy with standard regimens.

Studies with CGS 16949A

Recent studies by our group have concentrated upon the non-steroidal aromatase inhibitor, CGS 16949A. This compound has a K_i of 0.17 nM when tested in human placental microsomes [1]. This compares with a K_i of 0.54 μM when studying aminoglutethimide in a similar fashion. Thus, CGS 16949A is approx. 2500-fold more potent than aminoglutethimide in this system. The rationale for our studies is that all non-steroidal aromatase inhibitors have a propensity to inhibit a wide range of cytochrome *P*-450-mediated steroid hydroxylations such as cholesterol side-chain cleavage, 18-hydroxylase, 11-hydroxylase, 21-hydroxylase and 17-hydroxylase as well as inhibiting aromatase [1]. A zone of optimal efficacy might then exist in which a certain amount of drug would block aromatase completely without inhibiting the other hydroxylation steps. We initiated a Phase I study with CGS 16949A to determine its optimal dose, establish its toxicity, demonstrate the efficacy of estrogen suppression, study the specificity of CGS 16949A as an aromatase inhibitor, and to evaluate its pharmacokinetics [11]. As shown in Fig. 1, the dose-ranging study initially utilized 0.6 mg of CGS daily and escalated the dosage at 2-weekly intervals to 16 mg daily. Plasma and urinary estrogens were measured at 2-weekly intervals. As shown, the estrogens began to fall at the 0.6 mg daily dosage and appeared to plateau at dosage levels of 1.2–2 mg daily. Continued suppression was observed thereafter at doses up to 16 mg daily. The degree of suppression was approx. 60–70%. Urinary estrone levels fell from approx. 1.4 μg/24 h to levels of approx. 0.35 μg/24 h, urinary estradiol fell from 0.6 μg/24 h to approx. 0.2 μg/24 h. After obtaining these results, we questioned the specificity of radioimmunoassay measurements at

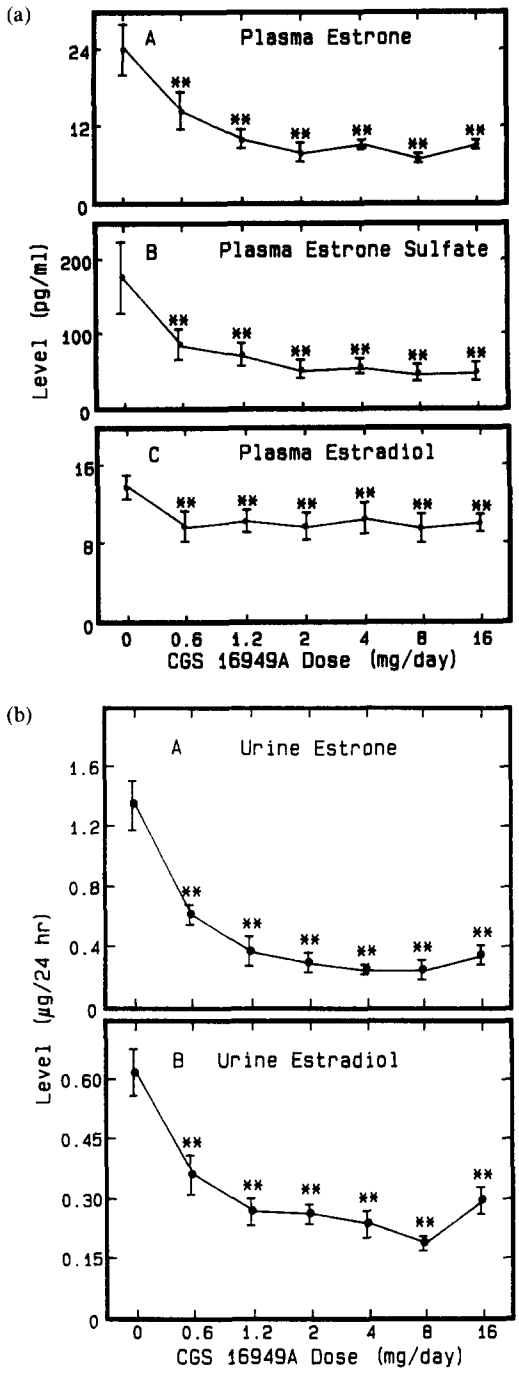

Fig. 1. Effects of CGS 16949A on levels of plasma and urine steroids. $*P < 0.05$, $**P < 0.01$. Doses were escalated at 2-weekly intervals. (Reproduced with permission from R. J. Santen *et al.* [11].)

such low concentrations in urine. To evaluate this further, studies were conducted in conjunction with Dr Herman Adlercreutz using GLC–mass spectrometry technology. Measurement of urinary estrone yielded similar results by radioimmunoassay or by GLC–mass spectrometry (Fig. 2). In contrast, levels of estradiol by radioimmunoassay reached approx. 0.2 μg/24 h, whereas those measured by GLC–mass spectrometry averaged approx. 0.05 μg/24 h. From these studies, we concluded that the degree of estradiol suppression with CGS 16949A was greater than indicated by measurement of urinary estradiol by radioimmunoassay.

After demonstrating inhibition of estrone and estradiol levels, we wished to examine the degree of specificity of inhibition with CGS 16949A. Prior *in vitro* studies demonstrated no inhibition of cholesterol side-chain cleavage. Consequently, we examined the possibility that inhibition of the conversion of 17α-hydroxyprogesterone to cortisol might take place. Indirect evidence of such an inhibition consists of a concordant increase in 17-hydroxyprogesterone, androstenedione and testosterone in plasma. Indeed, in patients receiving 4–16 mg daily, there were substantial and significant rises in these three steroids. This would indicate a blockade either of the enzyme, 21-hydroxylase or of 11-hydroxylase. Initial data from our laboratory including basal measurements of 11-deoxycortisol did not support blockade of the 11-hydroxylase step and we concluded that 21-hydroxylase was affected. However, Dr Steven Lamberts conducted a detailed *in vitro* study demonstrating that CGS 16949A exhibited potent effects on 11β-hydroxylase [12]. Consequently, we measured the levels of 11-desoxycortisol under basal conditions and 30 and 60 min following administration of Cortrosyn®, and then compared these results with measurements made with patients on 1.8–4 mg of CGS daily. We found a minor rise in basal 11-desoxycortisol levels from 0.3 to 0.4 μg/day. Peak values 60 min after Cortrosyn® increased from

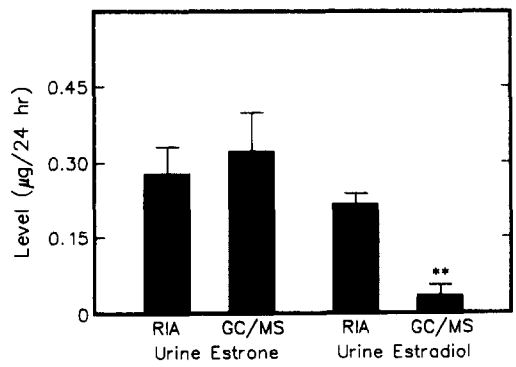

Fig. 2. Measurement of urinary estrone and estradiol by radioimmunoassay and by gas chromatography/mass spectrometry performed by Dr Herman Adlercreutz from the Department of Clinical Chemistry, University of Helsinki, Meilahati Hospital, Helsinki, Finland. $*P < 0.05$ $**P < 0.01$ (Data reprinted from R. J. Santen *et al.* [11].)

0.5 to 0.9 μg/day. These results clearly indicated an inhibition of the 11β-hydroxylase step. The inhibition is minor, however, since peak responses to 3 g of metyrapone result in peak 11-desoxycortisol levels of 10 μg% in normal subjects. Nonetheless, to evaluate this further, we measured the levels of urinary free cortisol and ACTH in subjects receiving 0.6–16 mg of CGS daily. There were no decrements in urinary free cortisol, even at the highest dose of medication. However, variable increases in ACTH were observed at 4 and 16 mg daily, suggesting a minor blockade of cortisol biosynthesis with a compensatory reflex rise in ACTH.

Systematic examination of additional steroids revealed a reduction in plasma aldosterone at the 8 and 16 mg daily doses of CGS 16949A [13]. This reduction could result from a blockade of 11β-hydroxylase or alternatively, from distal blocking effects on the aldosterone biosynthetic pathway. Based upon our experience in patients with corticosterone methyloxidase type II (CMO 11) deficiency, we initially suspected this step as the mechanism of action of CGS 16949A. Such inhibition would result in a lowering of plasma aldosterone with an increase of 18-hydroxycorticosterone. In collaboration with Dr James Melby, we measured these plasma steroids as well as their urinary analogs, tetrahydroaldosterone and tetrahydroxy-18-hydroxy-11-corticosterone. The reduction in aldosterone and its metabolites with an increase in 18-hydroxycorticosterone and its urinary metabolites establish that CGS 16949A does, in fact, block corticosterone methyloxidase type II [13]. The rise in plasma 18-hydroxycorticosterone would provide evidence that the 11β-hydroxylase step is not major in producing aldosterone inhibition. To our knowledge, CGS 16949A is the only compound yet described to block the CMO-II enzyme.

In the Phase I trial, systematic examination of hematologic and biochemical parameters were conducted to establish non-toxicity. These data indicated that CGS 16949A is non-toxic and associated with minimal side effects [14].

After completion of the Phase I study, a multicenter Phase II study involving 56 patients was initiated [15]. Four centers, including The Pennsylvania State University, the Dana-Farber Cancer Center, the University of Tennessee and the Simon Williamson Clinic in Birmingham, Alabama were utilized for patient entry. The protocol involved randomization to regimens including 0.6 mg three times daily, 1 mg twice daily and 2 mg twice daily. Plasma and urinary steroids were measured at weeks 2, 4, 6, 8, 10, 12 and then at 3-monthly intervals thereafter for responders of patients with stable disease. The primary goal of the study was to demonstrate potential dose response differences among the three regimens. Upon examination of the data, a similar degree of suppression of plasma and urinary estrogens was observed with each of the three dose schedules. No statistically significant differences among each dose could be demonstrated. These data allow the conclusion that a 1 mg b.i.d. dosage appeared optimal, that a three times daily dose schedule was not necessary, and that no greater suppression could be demonstrated with the 2 mg twice daily dosage. With respect to specificity, no statistically significant reduction of plasma cortisol, urinary free cortisol, nor plasma aldosterone could be detected under basal conditions. However, measurement of plasma aldosterone and cortisol after Cortrosyn® uncovered inhibitory effects of CGS 16949A at each of the three doses. Peak cortisol levels at 60 min after Cortrosyn fell by approx. 5 μg% at all dose levels during each visit on therapy. In 94% of tests, plasma cortisol increased to above 20 μg% or exhibited a rise of at least 7 μg% over basal. These criteria predict normal responses to severe stress such as the induction of surgical anesthesia. In contrast, responses of plasma aldosterone to cortrosyn were markedly and statistically significantly blunted. Measurement of plasma sodium and potassium concentrations, urinary sodium and potassium concentrations, and standing and lying blood pressures suggested that symptomatic mineralocorticoid deficiency does not occur in patients receiving either of the three dose regimens. Further studies, however, are required to determine the significance of the relative inhibition of mineralocorticoid biosynthesis.

After demonstration of estrogen suppression with CGS 16949A, the question of absolute degree of suppression was addressed. One means to evaluate this issue was to compare the degree of suppression of plasma and urine estrogens with CGS 16949A, 2 mg daily, with that observed previously with aminoglutethimide, 1000 mg daily, and 40 mg of hydrocortisone daily. The degree of suppression of plasma estrone, estrone sulfate, estradiol and urinary estrone and estradiol appeared similar with the two regimens. Since the regimen of amino-

glutethimide and hydrocortisone induces breast tumor regressions with a similar frequency as tamoxifen in randomized trials, these data would support the conclusion that CGS 16949A causes a major inhibition of estrogen biosynthesis.

To evaluate this issue in more detail, isotopic kinetic studies were then conducted. The most sensitive means of assessing aromatase inhibition involves the administration of [^{3}H]-androstenedione along with [^{14}C]estrone as a recovery marker before and during CGS 16949A therapy. This methodology provides a highly sensitive and specific means of assessing total body aromatization. Since no response differences in plasma and urinary estrogens were observed among the 0.6 tid, 1 bid and 2 bid doses of CGS, it appeared appropriate to pool results from patients on all three doses of CGS. The percentage conversion of androstenedione to estrone during treatment was 0.42%. These results indicate that inhibition of aromatase is approx. 85% with the doses of CGS 16949A utilized. This is to be compared with the 90–95% inhibition of aromatase observed previously with standard doses of aminoglutethimide and the absolute inhibition of aromatase to approx. 0.2% with 250 mg of aminoglutethimide twice daily [16, 17]. Further dose–response studies utilizing this highly sensitive technique are necessary to establish the absolute degree of suppression of aromatase with CGS 16949A. Preliminary data from Dowsett *et al.* (presented at the Rotterdam meeting) indicated in three patients that 4 mg of CGS 16949A produces a greater level of suppression of aromatase than 1 mg twice daily.

Preliminary data on clinical responses to CGS 16949A are available. A total of 56 patients have been entered into the Phase II study. These women have been heavily pretreated prior to entry into study. Twenty-seven patients had previously received both chemo- and hormonal therapy, six chemotherapy alone, 15 hormone therapy alone, and six no prior therapy. With respect to the number of previous hormonal therapies, five patients had received four hormonal therapies, eight received three, 13 received two, 16 received one and 14 no prior hormonal treatments. Preliminary evaluation of results are available in 54 evaluable patients. Of these, three experienced a complete objective tumor regression, eight partial regression for a total objective response rate of 20%. Twenty-five patients exhibited no change in measurable tumor for at least 6 wk and 18 exhibited tumor progression. The mean duration of responses of these for CR and PR was 14 months with a range of 17–18+ months. Sites of response predominantly included soft tissue and bone.

CONCLUSIONS

Reviewing recently available data, we can conclude that highly-potent, specific and non-toxic aromatase inhibitors are becoming available for clinical testing. These agents should be ideal for the treatment of estrogen-dependent breast cancer in postmenopausal women because of lack of estrogen agonistic effects and lack of cross-resistance with the antiestrogens. Detailed comparisons will be necessary between the mechanism-based or "suicide" inhibitors and the competitive inhibitors. Further, comparisons of the steroidal with non-steroidal inhibitors will also be required to determine the ideal aromatase inhibitor to be utilized clinically. This area is currently under intense study and new data are rapidly becoming available. Maturation of phase III studies will be required before determination of the relative phase of aromatase inhibitors in the therapeutic armamentarium for advanced breast cancer.

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