Journal of Cellular Biochemistry 17G:242–246 (1993)

Clinical Use of Aromatase Inhibitors in the Treatment of Breast Cancers

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Abstract Estrogens are the major hormones supporting the growth of human breast cancer. Aromatization of androgen precursors in peripheral tissues, including the breast cancer itself, is the major source of estrogens in postmenopausal women. Therefore, inhibition of the aromatase enzyme offers an effective means of inducing regression of hormone-responsive breast cancer. Aminoglutethimide, the first and most widely tested aromatase inhibitor, suppresses estrogen production to the level of adrenalectomy and exerts an anti-tumor action comparable to other standard endocrine therapies such as tamoxifen. However, conventional doses of the drug (1000 mg daily) cause moderate toxicity and inhibit other critical cytochrome P-450 steroidogenic enzymes, thus requiring concomitant glucocorticoid administration. New non-steroidal, competitive aromatase inhibitors with greater selectivity and less toxicity are being developed. The second generation compound, fadrazole (CGS 16949), lowers estrogen production to a degree similar to aminoglutethimide (50–80%), but at much lower doses (~2 mg daily) and is associated with minimal toxicity. Although not totally specific, this drug is sufficiently selective not to require simultaneous cortisol replacement. CGS 16949 has been shown to possess significant anti-tumor action in pilot studies and is currently being tested in Phase III trials. Recently, a third generation inhibitor, CGS 20267, has been found to have virtually complete selectivity for the aromatase enzyme. Furthermore, this drug suppresses estrogen biosynthesis to a greater extent (~90%) than previously observed with other aromatase inhibitors. Such enhanced activity may lead to a superior anti-tumor action, and may extend the use of this drug to a variety of other conditions where optimal suppression of estrogen biosynthesis is desired.

Key words: Aromatase inhibitors, breast cancer, estrogens

Estradiol is recognized as the predominant hormone supporting the growth of hormone-responsive human breast cancer. While the ovary is the principle source of estrogens in premenopausal women, aromatization of adrenal androgen precursors in peripheral tissues, including the breast cancer itself, is the major contributor to estrogen production in postmenopausal patients [1,2]. Therefore, the aromatase enzyme plays a critical role in the growth of hormone-responsive breast cancer by creating an estrogen-replete milieu. Furthermore, aromatase activity has been shown to be higher in adipose tissue adjacent to malignant tumors than in tissue closed to benign breast lesions [3]. This finding raises the possibility that regionally enhanced aromatase activity in the breast may produce a local environment conducive to mammary tumorigenesis.

Considerable effort has gone into developing potent inhibitors of aromatase activity as anti-tumor agents in breast cancer. Table I outlines the classification of these compounds based on

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TABLE I. Classification of Aromatase Inhibitors

Class	Mechanisms of Action	Advantages	Disadvantages	Examples
• Steroidal -competitive	Reversible binding to the enzyme based on structural similarity to testosterone and androstenedione	Specificity	-Hormone agonist effects -Crossreactivity in steroid RIAs	None in routine clinical use
-"suicide" or "mechanism-based"	Covalent binding with irreversible inactivation	Specificity		4-OH-androstenedione
• Non-steroidal competitive	Compete for substrate at the active enzyme site*	-Lack of hormone agonist effects -Lack of cross-reactivity in steroid RIAs	Binding to other cytochrome P-450 steroidogenic enzymes and inhibition of other hydroxylations (lack of specificity)	1st generation -Aminoglutethimide 2nd generation -Fadrazole (CGS 16949) 3rd generation -CGS 20267

*Unique three-dimensional architecture (if only preliminary information available since the enzyme is not crystallized). Structure-function studies have led to more potent and specific inhibitors.

their mechanism of action and lists their major advantages and disadvantages.

FIRST GENERATION INHIBITORS

Aminoglutethimide is the first and most widely tested aromatase inhibitor in clinical breast cancer treatment trials. It has been found to suppress estrogen biosynthesis as well as adrenalectomy, thus obviating the need for this major surgical procedure [4]. Furthermore, in randomized clinical trials, the anti-tumor action of aminoglutethimide has been equivalent to that of other optimal endocrine therapies, such as antiestrogens [5]. Aminoglutethimide, because of its greater toxicity—including lethargy, skin rash, orthostatic dizziness, and ataxia—is currently considered a secondary form of endocrine therapy in postmenopausal women, following initial treatment with tamoxifen and possibly progestins. Aminoglutethimide has also been tested as an adjuvant treatment for two years in 354 postmenopausal women with positive axillary lymph nodes [6]. After a median follow-up of 8.1 years, no prolongation of either event-free survival or overall survival was observed. These results are at variance with the beneficial effects observed with antiestrogen therapy under similar circumstances [7]. Finally, a significant drawback of aminoglutethimide is its lack of specificity, leading to inhibition of other steroidogenic enzymes such as the cholesterol side-chain cleavage enzymes, 21-hydroxylase, 11β-hydroxylase, and 18-hydroxylase. Therefore, patients treated with this drug also need glucocorticoid replacement. To increase specificity for aromatase and reduce toxicity, lower-than-conventional doses (1000 mg/day) of aminoglutethimide have been tried with some success. In a recent randomized study, 500 mg of aminoglutethimide with and without hydrocortisone were found to be equally as effective as a first-line endocrine treatment in postmenopausal women with metastatic breast cancer [8]. Side effects were infrequent and mild in both arms of the study. Specifically, no clinical episodes of adrenal insufficiency were observed; however, no endocrine testing was performed in that trial.

SECOND GENERATION INHIBITORS

Because of aminoglutethimide's lack of specificity and significant toxicity, considerable effort has gone into developing more potent and better-tolerated aromatase inhibitors. Among these, CGS 16949 (fadrazole) has been extensively tested. At concentrations of approximately 2 mg daily, this drug effectively blocks aromatase and does not produce clinically significant inhibition of cortisol and aldosterone biosynthesis [9]. However, upon careful endocrine testing, biochemical evidence of blockade of the 11β-hydroxylase and corticosterone methyloxidase type II enzymes has been demonstrated [9]. Pilot studies have shown anti-tumor activity of CGS 16949 in the absence of significant clinical toxicity [10]. Trials to assess the relative clinical efficacy of this drug versus other aromatase inhibitors, antiestrogens, and progestins are currently ongoing. In view of its greater selectivity and reduced toxicity, the development of CGS 16949 represents a definite improvement over aminoglutethimide in the clinical management of breast cancer. However, the degree of estrogen suppression induced by this compound has not been shown to be increased compared to aminoglutethimide [9]. These first and second generation aromatase inhibitors are limited because they block estrogen production by only 50–80%. Dose escalation to achieve a greater degree of inhibition is limited by drug side effects or lack of specificity for aromatase.

THIRD GENERATION INHIBITORS

CGS 20267 appears to be a particularly interesting third generation inhibitor. Despite a reduced affinity for aromatase *in vitro* compared to fadrozole [11], CGS 20267 is 10-fold more potent *in vivo* in the rat (Table II) [12] and 100-fold more potent in patients. This finding is due to the longer half-life of this compound *in vivo* compared to fadrozole [13]. After a single dose of CGS 20267 (0.1, 0.5, or 2.5 mg) in healthy postmenopausal women, a similar long-lasting suppression (approximately 80%) of serum estrone and estradiol was observed [13]. Neither estrone nor estradiol serum levels returned to baseline levels 14 days after the administration of the single dose. It should be noted that most of the measurements were below the sensitivity of the radioimmunoassay. Therefore, it is likely that this study underestimated the degree of estrogen suppression induced by this inhibitor. Serum levels of cortisol, aldosterone, 17-hydroxy-pro-

TABLE II. Comparison of the Potencies of CGS 20267 and Other Aromatase Inhibitors Using Microsomal Preparations of Human Placental Aromatase and an Assay of Androstenedione-induced Uterine Hypertrophy*

Compound	Inhibition of human placental aromatase *in vitro*		Inhibition of androstenedione-induced uterine hypertrophy	
	IC_{50} (nM)	Relative Potency	ED_{50} (μg/kg)	Relative Potency
Aminoglutethimide	1900	1	30,000	1
4-OH-androstenedione	62	30		
Fadrozole	5	380	30	1000
CGS 20267	11.5	165	1–3	10,000

*[13], with permission of the publisher

gesterone, androstenedione, testosterone, follicular stimulating hormone, luteinizing hormone, and thyroid stimulating hormone were not affected, thus indicating the high specificity of the drug [13]. CGS 20267 has also been tested in a Phase I study in postmenopausal patients with advanced breast cancer [14]. A similar suppression of estrone and estradiol was observed upon chronic administration of the drug. As in the previous study, no difference in the degree of estrogen suppression was observed following treatment with 0.1, 0.5, or 2.5 mg. CGS 20267 was again found to be highly selective for the aromatase enzyme and devoid of major clinical toxicity. The main side effects were headache in six patients and gastrointestinal symptoms in five out of 21 treated patients. Objective tumor responses were also observed in one-third (7/21) of the women (one complete response and six partial responses) [14].

A central question is to what extent the availability of third generation non-steroidal aromatase inhibitors will improve the treatment of breast cancer. The observed sequential responses of endocrine-dependent tumors to stepwise reduction in circulating estrogen levels (such as following sequential therapy with ovariectomy and aromatase inhibitors) suggest that breast tumors may adapt to a low estradiol milieu by enhancing their estrogen sensitivity. Therefore, more profound suppression of estrogen production with potent aromatase inhibitors could produce further tumor regression in these patients. In addition, development of potent aromatase inhibitors (as well as pure antiestrogens) may be useful to treat those patients whose tumors have become sensitive to the agonistic properties of tamoxifen, the currently used antiestrogen. First generation aromatase inhibitors, such as aminoglutethimide, are unable to suppress ovarian estrogen biosynthesis in premenopausal women [15]. This failure is felt to be due to the reflex increase in gonadotropins which overrides the enzymatic blockade. Whether third generation inhibitors, by virtue of their higher potency, will be able to induce a medical ovariectomy remains to be established. If this is the case, drugs such as CGS 20267 may find a role in the treatment of a variety of other conditions, both central and ovarian in origin, such as dysfunctional uterine bleeding and precocious puberty.

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