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New agents in breast cancer — minisymposium

Aromatase inhibitor development for treatment of breast cancer

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Summary

Inhibition of estrogen production provides effective therapy for patients with hormone-dependent breast cancer. The source of estrogens in premenopausal women is predominantly the ovary, but after the menopause, estradiol is synthesized in peripheral tissues through the aromatization of androgens to estrogens. Uptake from plasma is the primary mechanism for maintenance of estradiol concentrations in breast cancer tissue in premenopausal women, whereas several steps may be operant in postmenopausal women. These include enzymatic synthesis of estradiol via sulfatase, aromatase, and 17β-hydroxysteroid dehydrogenase in the tumor itself. Aromatization of androgens secreted by the adrenal to estrogens in peripheral tissues and transport to the tumor via circulation in the plasma provides another means of maintaining breast tumor estradiol levels in postmenopausal women. These various sources contribute to the high tissue estrogen levels measured in breast tumor tissue.

To effectively suppress tissue concentrations of estrogens and circulating estradiol in postmenopausal patients, various aromatase inhibitors have been developed recently. These include steroidal inhibitors such as 4-hydroxy-androstenedione as well as non-steroidal compounds with imidazole and triazole structures. The most potent of these, CGS 20267, is reported to suppress levels of active estrogens (i.e., estrone, estrone sulfatase, and estradiol) by more than 95%. This compound can suppress both serum and 24-hr urine estrogens to a greater extent than produced by the second generation inhibitor, CGS 16949A. CGS 20267 is highly specific since it does not affect cortisol and aldosterone serum levels during ACTH stimulation tests nor sodium and potassium balance in 24-hr urine samples. These data suggest that CGS 20267 can be expected to bring improved response rates in the treatment of metastatic hormone-dependent breast cancer without substantial side effects.

Introduction

A subpopulation of human breast cancers are dependent upon estradiol for cellular proliferation. Studies to elucidate the mechanism of estradiol stimulated growth have been ongoing for two

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decades. Several hormonally related strategies have been developed for treatment of human breast cancer which are based upon the principle that estrogens are mitogenic for these tumor cells. Initial methods involved surgical ablative therapies such as oophorectomy, adrenalectomy, and hypophysectomy. These procedures eliminate ovarian estrogen synthesis, adrenal steroid synthesis, and the stimulatory effects of the gonadotropins on estrogen production in the ovaries, respectively. The rates of response to these therapies range from 30-40% [1].

Adrenalectomy and hypophysectomy, because they involve major surgery, are infrequently employed currently, whereas oophorectomy continues to be selected. Pharmacologic methods to alter the hormonal milieu eliminate the need for major surgery and have generally replaced surgical ablative therapies. Agents currently used are the antiestrogen tamoxifen, the progestins medroxyprogesterone acetate and megestrol acetate, gonadotropin releasing hormone analogs such as goserelin, and inhibitors of estrogen biosynthesis such as the aromatase inhibitors. Surprisingly, inhibition of aromatase, which blocks the conversion of androgens to estrogens, is effective therapy in patients with breast cancer even after they relapse from responses to antiestrogen or progestin (medroxyprogesterone acetate or megestrol acetate) therapy. Several second- and third-generation aromatase inhibitors are now available which are highly potent and associated with few side effects. Their role in the therapy of breast cancer will probably become increasingly important. In this review, the current status of aromatase inhibitors will be discussed.

General role of aromatase

Fat, liver, muscle, and hair follicles contain the aromatase enzyme which catalyzes the conversion of androgens to estrogens [2,3]. In postmenopausal women, the major source of circulating estrogens is the peripheral conversion from androgens in fat tissue and in muscle [2]. Androstenedione, the major precursor androgen, and testosterone, a minor substrate, are secreted primarily from the adrenal glands and are converted in peripheral tissues to estrone and estradiol, respectively, through the catalytic action of the enzyme aromatase. The major aromatized product, estrone, is then enzymatically reduced to estradiol by the enzyme 17β-hydroxysteroid dehydrogenase. These enzymatic activities result in measurable amounts of circulating estrogen in the range of 10-20 pg/ml in the plasma of postmenopausal women.

Despite relatively low serum concentrations, the levels of estradiol in breast tumors of postmenopausal women are almost equivalent to those in premenopausal women [4]. The tumor tissue levels are much higher than the values predicted from calculations of serum concentrations and the affinity (K_d) of tissue receptors for estradiol. One of the explanations for maintenance of high tissue estradiol concentrations is the *in situ* synthesis of estradiol catalyzed by the various enzymatic activities present in breast tumor tissue itself. These include sulfatase which catalyzes the conversion of estrone sulfate to estrone, aromatase which mediates androgen to estrogen conversation, and 17β-hydroxysteroid dehydrogenase which allows formation of estradiol from estrone. The absolute levels of aromatase activity in human breast cancer tissues are low (5-100 pg/gm tissue/hr) when compared to those of sulfatase and 17β-hydroxysteroid dehydrogenase [5]. However, it is difficult to quantitate experimentally the amount of estradiol synthesized locally by each enzymatic pathway and the amount concentrated in tissue via uptake from plasma. Nonetheless, tissue enzymes such as sulfatase, 17β-hydroxysteroid dehydrogenase, and aromatase are likely to be involved in the production of at least some of the estrogen present *in situ* in tumor tissue. As another possible source of estrogens in breast tumors, lipoidal estradiol is reported to accumulate in estrogen receptor (ER) positive as well as ER negative breast cancer cells and can be hydrolyzed to free estradiol by esterase or lipase in tissues [6,7].

Androstenedione is the major circulating

Table 1. Partial list of aromatase inhibitors [20].

Type of inhibition	Type of compound	Name of compound	Ki[a]	K intact[b]
Mechanism based	Steroid	1,4,6-androsta-triene-3,17-dione		$1.1x10^{-3}S^{-1}$
	Steroid	4-OH-androstenedione		$4.5x10^{-3}S^{-1}$
	Steroid	4-androstene-3,6,17- trione		$4.03x10^{-3}S^{-1}$
	Steroid	Testolactone		$5.5x10^{-4}S^{-1}$
	Steroid	10β-propargylestr-4-ene-3,17-dione		$1.11x10^{-3}S^{-1}$
	Steroid	7α(4'-amino)phenylthio-1,4-androstadiene-3,17-dione		$8.4x10^{-3}S^{-1}$
	Steroid	1-methyl-androsta-1,4-diene-3,17-dione		$1.8x10^{-4}S^{-1}$
Competitive	Steroid	6α-bromo-androstene-dione	3.4nM	
	Steroid	7α(4'-amino)phenylthio-4-androstene-3,17-dione	18nM	
	Non-steroid	Aminoglutethimide	540nM	
	Non-steroid	Pyridoglutethimide	1100nM	
	Imidazole	CGS 16949A	0.19nM	
	Imidazole	R-76713	0.70nM	
	Imidazole	CGS 20267	—	
	Imidazole	Econazole	0.06μM[c]	

[a] Ki – inhibitory constant
[b] K inact – rate constant for inactivation of the enzyme (S=seconds)
[c] IC50

substrate for estrogens in the plasma of postmenopausal women and aromatase is the rate-limiting enzyme that regulates the conversion of androstenedione to estrone. Thus, the control of aromatase activity in tumors and in peripheral tissues can be a critical factor for regulation of postmenopausal breast cancer growth. Consequently, this enzyme provides a unique target for inhibition of tumor estradiol concentrations.

Development of aromatase inhibitors

The first aromatase inhibitor used in clinical studies was aminoglutethimide [8-10]. The rates of objective (complete or partial) tumor regression with this agent were equal to those induced by surgical ablative therapies, antiestrogens, or high doses of progestin [11]. Aminoglutethimide was also active in a substantial number of patients with breast cancer who exhibited total resistance to tamoxifen [12]. In spite of the clinical effectiveness and partial non-cross resistance with antiestrogens, aminoglutethimide is not an ideal aromatase inhibitor because of its non-specificity and side effects.

To overcome these drawbacks, more potent and specific aromatase inhibitors have been under development. As a class, these inhibitors can be divided into those which are steroidal and compete for the active site of the enzyme, and non-steroidal compounds which structurally fit into or near the enzyme active site. Further, some inhibitors are altered by the aromatase enzyme to make sites available which bind covalently to and permanently inactive the enzyme. Agents of this type are called mechanism-based or "suicide" inhibitors.

Pyridoglutethimide is one of the non-steroidal compounds developed through modification of the structure of aminoglutethimide to bring greater specificity and lesser side effects. While lacking the drawbacks of aminoglutethimide such as inhibition of cholesterol side-chain cleavage and sedative properties on the CNS [13,14], this compound is considerably less potent than its parent. Another non-steroidal competitive inhibitor, R-76713, is also highly potent and specific as an

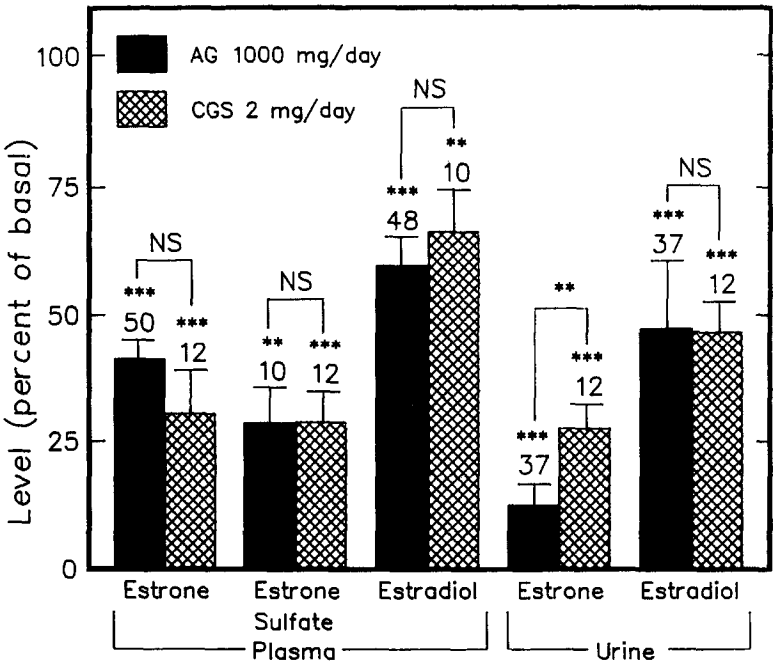

Figure 1. Estrogen levels in plasma and urine (mean ± SE), expressed as percent suppression from basal values (before treatment). In this study, postmenopausal breast cancer women are treated either with 1000 mg aminoglutethimide (AG) and 40 mg cortisol for 2-12 weeks or with 2 mg CGS 16949A. The numbers over the bars represent the number of patients analyzed. (**, P<0.01; ***, P<0.001; by paired comparison of basal and treatment values.)

aromatase inhibitor. R-76713 has the potency to suppress circulating estradiol to undetectable levels in normal men and to achieve 64% inhibition in premenopausal women [15,16]. While the racemic form was initially studied, the stereoisomer R83842 is more potent and is currently under clinical study [17].

4-hydroxyandrostenedione is one of the mechanism-based ("suicide") class of inhibitors. This compound effectively suppresses estrogen synthesis while associated with no estrogenic, antiestrogenic, or antiandrogenic properties [18]. Metabolism to 4-hydroxytestosterone renders it slightly androgenic but this does not appear to be associated with clinical sequelae. A large clinical trial conducted by Hoffken et al. [19] utilized 500 mg of 4-hydroxyandrostenedione administered intramuscularly every two weeks for six weeks, and then 250 mg every two weeks thereafter. This compound reduced plasma estradiol of postmenopausal patients from 10-11 pg/ml to 4 pg/ml for up to 7 months. 24% of women experienced an objective tumor responses while 11% noted minor side effects such as hot flashes or constipation. Other studies reported sterile abscesses due to the intramuscular injection required for administration of this compound [14]. However, this effect is apparently reduced by improved formulation. While oral administration of this agent might be considered preferable, this route is limited by the marked first-pass effect on metabolism in the liver and rapid production of glucuronidated derivatives.

Other non-steroidal aromatase inhibitors are being developed as well. CGS 16949A (fadrazole hydrochloride) and CGS 20267 represent two of these agents (Table 1) [13]. Our studies demonstrated that fadrazole is 500-fold more potent than aminoglutethimide, but not completely specific for aromatase (Figure 1). Fadrazole hydrochloride also blocks cortisol and aldosterone synthesis through inhibiting 11β-hydroxylase activity and corticosterone methyl oxidase [21]. While basal levels of circulating cortisol and aldosterone are

not significantly influenced, ACTH-stimulated cortisol and aldosterone responses are blunted, even when low doses (i.e., 1.8, 2.0, and 4.0 mg/day) of fadrazole hydrochloride are used. Blockade of 11β-hydroxylation by CGS 16949A also increases levels of precursor steroids such as 17α-hydroxyprogesterone and androstenedione in some patients.

We have recently directed our attention toward CGS 20267, an even more potent and specific aromatase inhibitor. CGS 20267 (letrozole) is a compound of the benzonitrile class which is approximately 1,000- to 10,000-fold more potent than aminoglutethimide and approximately 8-fold more potent than fadrazole hydrochloride. We initially studied 8 postmenopausal patients with advanced breast cancer who received 0.1 mg/day of CGS 20267 for 6 weeks, followed by 0.25 mg/day for an additional 6 weeks. Greater than 90% suppression of estradiol, estrone, and estrone sulfate were achieved over a 2-week period and the patients reached over 97% suppression by 6 weeks of therapy with the dose of 0.1 mg/ml (Figure 2) [22]. Since very low levels of circulating estrogens are present in these patients, standard radioimmunoassays are not sufficiently sensitive for the assessment of aromatase inhibition under these circumstances [23]. For this reason, in our studies estradiol was measured with a highly sensitive assay combining a high specific activity trace with a high affinity antiserum obtained from Baker Clinical Assays (Germany). This radioimmunoassay provides a sensitivity of 0.1 pg/ml with a direct non-chromatographic method involving diethyl ether extraction of plasma [22]. The radioimmunoassay utilized for estrone measurements is also sensitive enough to detect levels as low as 1 pg/ml. To further substantiate the degree of estrogen suppression achieved with CGS 20267, we also utilized a highly sensitive technique involving gas liquid chromatography-mass spectrometry (GLC/MS) analysis of 24-hr urine specimens. Before and 12 weeks after treatment with three different doses of CGS 20267, 24-hr urines were collected and estrone, estradiol, estriol, catecholestrogens, and other estrogens were measured by the method of Fotsis and Adlercreutz [24]. A total of 13 postmenopausal breast cancer patients received CGS 20267 for 6 weeks in initial doses of 0.1-2.5 mg/day followed over a 6-week period by an increasing amount of the compound to 0.25-5 mg/day. Since there were no statistically significant differences in levels of suppression

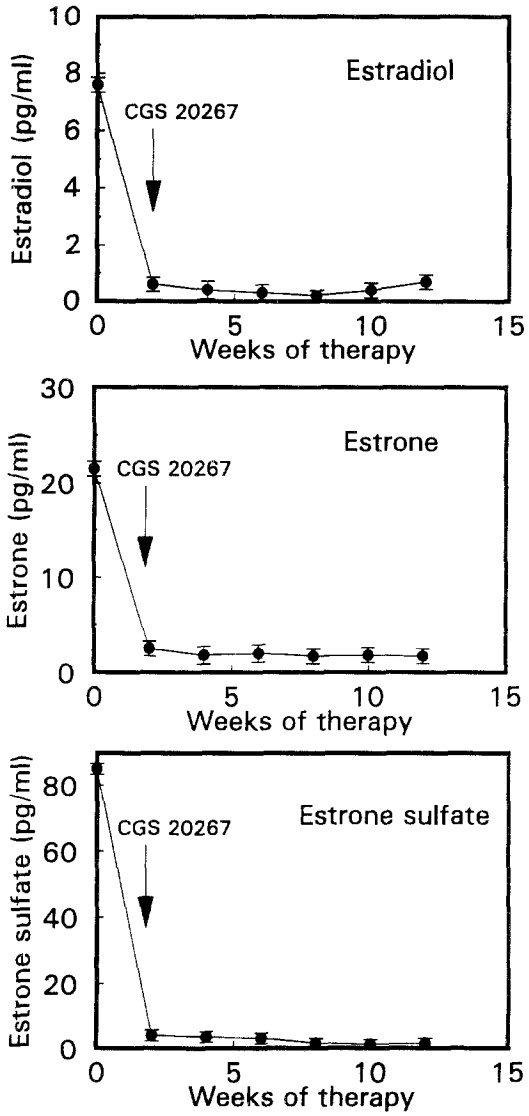

Figure 2. Effects of CGS 20267 on plasma estradiol, estrone, and estrone sulfate in 8 patients at doses of 0.1 mg/day (first 6 weeks) and 0.25 mg/day (second 6 weeks) over 12 weeks of therapy. Results represent mean ± SD. Data reproduced from ref. [22].

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