Aromatase and Its Inhibitors: Significance for Breast Cancer Therapy

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ABSTRACT

Endocrine adjuvant therapy for breast cancer in recent years has focussed primarily on the use of tamoxifen to inhibit the action of estrogen in the breast. The use of aromatase inhibitors has found much less favor due to poor efficacy and unsustainable side effects. Now, however, the situation is changing rapidly with the introduction of the so-called phase III inhibitors, which display high affinity and specificity towards aromatase. These compounds have been tested in a number of clinical settings and, almost without exception, are proving to be more effective than tamoxifen. They are being approved as first-line therapy for elderly women with advanced disease. In the future, they may well be used not only to treat young, postmenopausal women with early-onset disease but also in the chemoprevention setting. However, since these compounds inhibit the catalytic activity of aromatase, in principle, they will inhibit estrogen biosynthesis in every tissue location of aromatase, leading to fears of bone loss and possibly loss of cognitive function in these younger women. The concept of tissue-specific inhibition of aromatase expression is made possible by the fact that, in postmenopausal women when the ovaries cease to produce estrogen, estrogen functions primarily as a local paracrine and intracrine factor. Furthermore, due to the unique organization of tissue-specific promoters, regulation in each tissue site of expression is controlled by a unique set of regulatory factors. These factors are potential targets for the design of selective aromatase modulators, which could selectively inhibit aromatase expression in breast with the same efficacy as the phase III inhibitors of activity but leave expression in other local sites such as bone and brain untouched.

I. Aromatase and Its Gene

Estrogen biosynthesis is catalyzed by a microsomal member of the cytochrome P450 superfamily, namely, aromatase cytochrome P450 (P450arom, the product of the *CYP19* gene). The P450 gene superfamily is a very large one, containing (as of 1996) over 480 members in 74 families, of which cytochrome P450arom is the sole member of family 19 (Nelson *et al.*, 1996). This heme protein is responsible for binding of the C_{19} androgenic steroid substrate and catalyzing the series of reactions leading to formation of the phenolic A ring characteristic of estrogens.

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The human *CYP19* gene was cloned some years ago (Means *et al.*, 1989; Harada *et al.*, 1990; Toda *et al.*, 1990), when it was shown that coding region spans nine exons, beginning with exon II. Upstream of exon II are a number of alternative first exons that are spliced into the 5′-untranslated region of the transcript in a tissue-specific fashion (Figure 1). For example, placental transcripts contain at their 5′-end a distal exon, I.1. This is because placental expression is driven by a powerful distal promoter upstream of exon I.1 (Means *et al.*, 1991). Examination of Human Genome Project data reveals that exon I.1 is 89 kb upstream of exon II (Sebastian and Bulun, 2001). On the other hand, transcripts in ovary and testes contain at their 5′-end sequence that is immediately upstream of the translational start site. This is because expression of the gene in the gonads utilizes a proximal promoter, promoter II. By contrast, transcripts in cells of mesenchymal origin (e.g., adipose stromal cells, osteoblasts) contain yet another distal exon (I.4) located 20 kb downstream of exon I.1 (Mahendroo *et al.*, 1993). Adipose tissue transcripts also contain promoter II-specific exonic sequences that are undetectable in bone (Shozu and Simpson, 1998).

Splicing of these untranslated exons to form the mature transcript occurs at a common 3′-splice junction upstream of the translational start site. This means that although transcripts in different tissues have different 5′-termini, the coding region – and thus the protein expressed in these various tissue sites – is always the same. However, the promoter regions upstream of each of the several untranslated first exons have different cohorts of response elements, so regulation of aromatase expression in each tissue differs. Thus, the gonadal promoter (II) binds the transcription factors cyclic AMP (cAMP) response binding protein (CREB) and steroidogenic factor 1 (SF1), so aromatase expression in gonads is regulated by cAMP and gonadotrophins. In adipose tissue, promoter II-mediated expression is stimulated by prostaglandin E_2. On the other hand, promoter I.4 is regulated by class I cytokines such as interleukin (IL)-6, IL-11, and oncostatin M as well as by tumour necrosis factor alpha (TNFα). Thus, the regulation of estrogen biosynthesis in each tissue site of expression is unique (reviewed in Simpson *et al.*, 1997). This situation leads to a complex physiological situation that makes, for example, interpretation of circulating estrogen levels as a marker of aromatase activity in specific tissues or in response to specific stimuli very difficult.

II. The Concept of Local Estrogen Biosynthesis

In premenopausal women, the ovaries are the principal source of estrogen, which functions as a circulating hormone to act on distal target tissues. However, this is no longer the case in postmenopausal women, when the ovaries cease to produce estrogens, and in men. Under these circumstances, estradiol is no longer
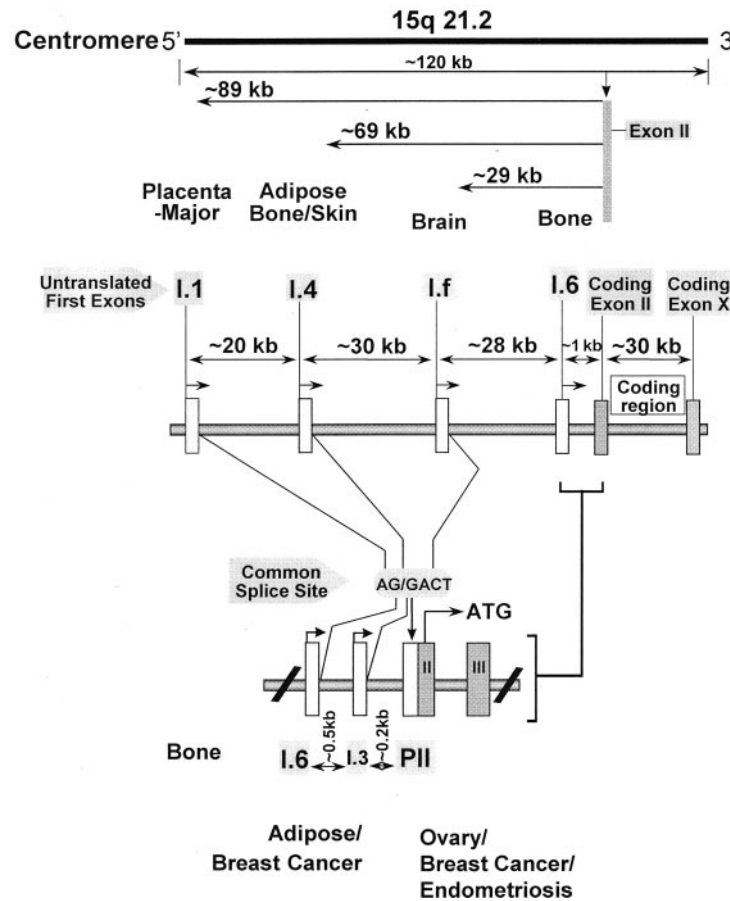
FIG. 1. Genomic organization of the human *CYP19* gene. BLAST searches of various promoters and coding region revealed alignment to distinct locations in two overlapping BAC clones of chromosome 15q 21.2 region. The distance of each promoter with respect to the first coding exon (exon II) also was determined. The major placental promoter I.1 is the most distally located (approximately 89 kb). Even though each tissue expresses a unique untranslated first exon 5′-untranslated region (UTR), by splicing into a highly promiscuous splice acceptor site (AG/A\ACT) of exon II, the coding region and the translated protein is identical in all tissue sites of expression. [Adapted with permission from Sebastian S, Bulun SE 2001 A highly complex organization of the regulatory region of the human CYP19 (aromatase) gene revealed by the human genome project. J Clin Endocrinol Metab 86:4600–4602. Copyright The Endocrine Society.]

solely an endocrine factor. Instead, it is produced in a number of extragonadal sites where it acts locally, including the mesenchymal cells of adipose tissue, osteoblasts and chondrocytes of bone, the vascular endothelium and aortic

smooth muscle cells, and numerous sites in the brain. Thus, circulating levels of estrogens in postmenopausal women and in men are not the sole drivers of estrogen action: they may be predominantly reactive rather than proactive. This is because circulating estrogen in this situation originates in extragonadal sites, where it acts locally. If it escapes local metabolism, it enters the circulation. Therefore, circulating levels in large part reflect, rather than direct, estrogen action in postmenopausal women and in men.

Extragonadal sites of estrogen biosynthesis possess several fundamental features that differ from those of the ovaries. First, the estrogen synthesized within these compartments acts predominantly at the local tissue level in a paracrine or intracrine fashion (Labrie *et al.*, 1997,1998) (Figure 2). The total amount of estrogen synthesized by these extragonadal sites may be small but the local tissue concentrations achieved are probably high and exert biological influence locally. As a consequence, extragonadal estrogen biosynthesis plays an important but hitherto largely unrecognised physiological and pathophysiological role.

The power of local estrogen biosynthesis is illustrated in the case of postmenopausal breast cancer (Pasqualini *et al.*, 1996). It has been determined that the concentration of estradiol present in breast tumours of postmenopausal women is at least 20-fold greater than that present in the plasma. With aromatase inhibitor therapy, intratumoural concentrations of estradiol and estrone drop precipitously, together with a corresponding loss of intratumoural aromatase activity, consistent with this activity being within the tumour and the surrounding
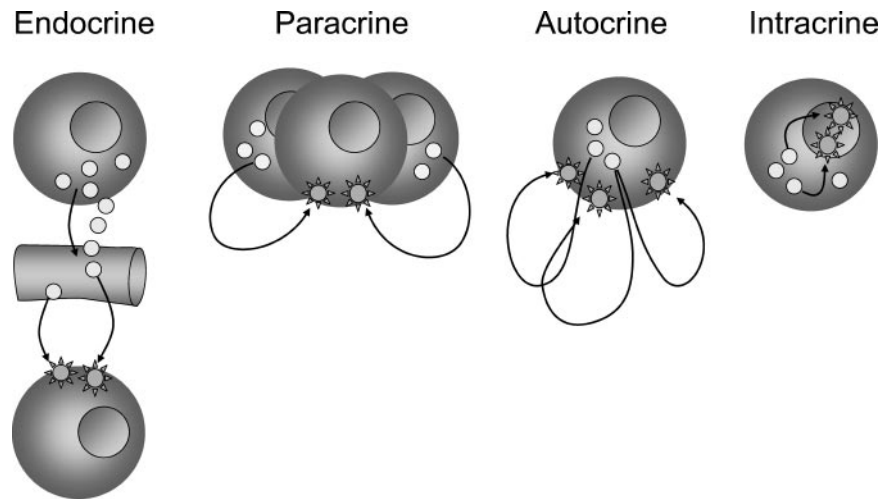

FIG. 2. Modes of action of endocrine, paracrine, autocrine, and intracrine factors.

breast adipose tissue and being responsible for these high tissue concentrations (DeJong *et al.*, 1997). Studies to assess directly the proportion of estrogen that is synthesized in breast tumours as opposed to reaching them from the blood are technically difficult but particularly instructive. The studies of Reed and colleagues as well as of Miller (Reed *et al.*, 1989; Miller, 1999) indicate that the majority of breast tumours generate estrogen through the intratumoural route. However, this varies substantially between tumours, and overall, about half of the estrogen is produced locally within the tumour or surrounding tissue (Miller, 1999).

In bone, aromatase is expressed primarily in osteoblasts and chondrocytes (Sasano *et al.*, 1997). Aromatase activity in cultured osteoblasts is comparable to that present in adipose stromal cells (Shozu and Simpson, 1998). Thus, it appears that in bone also, local aromatase expression is a major source of estrogen responsible for the maintenance of mineralization (although this is extremely difficult to prove due to sampling problems). Hence, for both breast tumours and bone, it is likely that circulating estrogen levels are only partly responsible for the relatively high endogenous tissue estrogen levels. The circulating levels reflect the sum of local formation in its various sites. This is a fundamental concept for interpreting relationships between circulating estrogen levels in postmenopausal women and estrogen insufficiency in specific tissues.

The second important point is that estrogen production in these extragonadal sites is dependent on an external source of C_{19} androgenic precursors, since these extragonadal tissues are incapable of converting cholesterol to the C_{19} steroids (Labrie *et al.*, 1997a,1998). As a consequence, circulating levels of testosterone and androstenedione as well as dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS) become extremely important in terms of providing adequate substrate for estrogen biosynthesis in these sites.

It should be pointed out that, in the postmenopausal woman, circulating testosterone and androstenedione levels are an order of magnitude greater than circulating estradiol and estrone levels. Differences in the levels of circulating androgens are likely to be important determinants for maintenance of local estrogen levels in extragonadal sites. Moreover, in men, circulating testosterone levels are an order of magnitude greater than those in postmenopausal women. In postmenopausal women, the ovaries secrete 25–35% of the circulating testosterone. The remainder is formed peripherally from androstenedione and DHEA produced in the ovaries and from androstenedione, DHEA, and DHEAS secreted by the adrenals. However, the secretion of these steroids and their plasma concentrations decrease markedly with advancing age (Labrie *et al.*, 1997b).

In this context, it is appropriate to consider why osteoporosis is more common in women than in men and why it affects women at a younger age in terms of fracture incidence. We have suggested that uninterrupted sufficiency of circulating testosterone in men throughout life supports the local production of

estradiol by aromatization of testosterone in estrogen-dependent tissues. This affords ongoing protection against the so-called estrogen deficiency diseases. This appears to be important in terms of protecting the bones of men against mineral loss and also may contribute to the maintenance of cognitive function and prevention of Alzheimer's disease (Simpson *et al.*, 2000).

III. Aromatase Inhibitors

A. BACKGROUND

A large number of aromatase inhibitors have been developed and utilised in clinical studies over the last 20 years. The most successful are now being licensed mainly for breast cancer treatment (Buzdar and Howell, 2001; Goss and Strasser, 2001).

This development was prompted by the recognition that the cytochrome P450 inhibitor aminoglutethimide is an aromatase inhibitor (Thompson and Siiteri, 1974) and exerts its therapeutic effectiveness in postmenopausal women with advanced breast cancer via the inhibition of aromatase (Santen *et al.*, 1978; Stuart-Harris *et al.*, 1984). This recognition validated aromatase as a new target for treatment of breast cancer patients with hormone-dependent disease. The widespread acceptance that aminoglutethimide was anything but a perfect drug (e.g., significant clinical side effects, incomplete inhibition of aromatase, poor selectivity for aromatase leading to adrenal suppression) and the need for combination with glucocorticoid led to the development of numerous new drugs. These have generally been categorised as first-, second-, and third-generation inhibitors. Figure 3 categorises the structures of a selection of the most prominent. Aminoglutethimide is recognised as the dominant first-generation compound. The second generation showed improved potency but either metabolic or symptomatic side effects limited the dose at which these inhibitors could be used. Therefore, overall pharmacological efficacy of the compounds was no greater than that of aminoglutethimide itself (Boeddinghaus and Dowsett, 2001). In contrast, the third-generation compounds have been found to be highly specific and well tolerated such that they have been usable at dosages that effectively obliterate the activity of aromatase.

Many pathological states at least partly depend on continued estrogen stimulation and, thus, in principle, might be expected to be good targets for aromatase inhibition. However, most of these diseases (e.g., endometriosis, fibroids) are almost entirely limited to premenopausal women and are not subject to targeting with aromatase inhibitors alone. Thus, the clinical application of inhibitors has been confined almost entirely to the main estrogen-dependent disease in postmenopausal women (i.e., breast cancer).
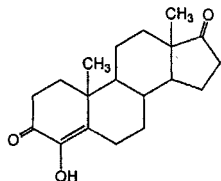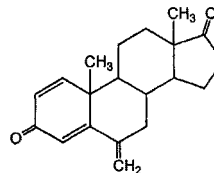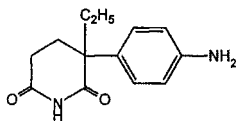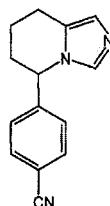
Steroidal Inhibitors
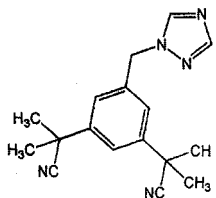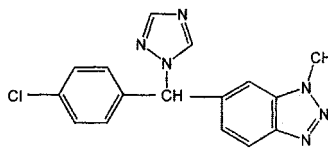
Formestane   **Exemestane**

Non-steroidal Inhibitors

Aminoglutethimide   **Fadrozole**

Anastrozole   **Vorozole**   **Letrozole**
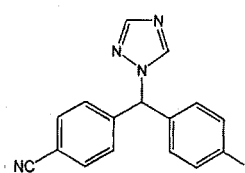
FIG. 3. Chemical structures of the most widely used aromatase inhibitors.

B. STRUCTURE AND BASIS OF INHIBITION

There are two main structural types of aromatase inhibitor: 1) steroidal, substrate analogs such as 4-hydroxyandrostenedione (formestane) and exemestane, and 2) nonsteroidal compounds that operate by virtue of their interaction with the cytochrome P450 heme prosthetic group of the aromatase enzyme (Kao *et al.*, 1996). These are known as type I and type II inhibitors, respectively. While the steroidal group includes inhibitors that act in a competitive manner, those of

greatest contemporary interest and clinical importance act as enzyme inactivators or suicide inhibitors (Brodie *et al.*, 1981; Lonning, 2000). This activity requires that the enzyme itself converts the inhibitor to a chemically reactive intermediate that binds irreversibly and covalently to the protein structure of the enzyme-substrate binding site. Thus, the individual enzyme molecule is irreversibly inactivated and the inhibitor molecule is no longer available to interact with other enzyme molecules. These types of inhibitors have the potential for exquisite selectivity for the enzyme target and long-term effectiveness, since the recovery of enzyme activity depends on the re-synthesis of enzyme as well as on the pharmacokinetics of the drug. However, such steroidal structures also have the potential for hormonal activity. The potential advantage of long-term effectiveness is of little importance when the turnover of enzyme is rapid. It is notable that plasma estradiol levels rise relatively rapidly after cessation of orally administered formestane (Dowsett *et al.*, 1987). This suggests that the aromatase enzyme is replenished in peripheral tissues within about 24 hours. Thus, this inactivation type of mechanism has yet to be demonstrated to be of significant clinical advantage (see below).

All type 2 inhibitors have a basic nitrogen atom that allows them to interact with the iron atom of the heme prosthetic group of the enzyme (Kao *et al.*, 1996). Their specificity for inhibition of the aromatase enzyme (as opposed to the very large number of other cytochrome P450 enzymes) is determined by the other structural aspects of the drugs and the way that these allow a close fit to the substrate-binding site of aromatase. This results in high-affinity binding and limits the fit into the substrate-binding site of other enzymes. A full understanding of these molecular interactions has been restricted by the unavailability of a crystallized aromatase preparation for structural analysis of the inhibitor-enzyme interaction. Thus, computer-generated models have depended largely on the structural analogies that can be surmised between aromatase and the few cytochrome P450 enzymes whose structure has been determined (Graham-Lorence *et al.*, 1995; Graham-Lorence and Peterson, 1996). Use of such models has illustrated the much-better fit to the substrate-binding site of aromatase by the triazole compounds anastrozole, letrozole, and vorozole than by aminoglutethimide, with letrozole and vorozole apparently having a somewhat more complete space-filling effect (Kao *et al.*, 1996).

The potency of these drugs generally has been assessed *in vitro* using human placental microsomal aromatase preparations. In this type of assay, fadrozole is one of the most potent drugs known, having an IC50 of 5 nM. However, its potency *in vivo* has been compromised by very rapid metabolism such that its *in vitro* activity has not been matched in *in vivo* studies (Bhatnagar *et al.*, 2001). Letrozole and anastrozole appear to have relatively similar IC50s to each other. But when these compounds have been tested on intact cells – including hamster ovarian tissue, human breast fibroblasts, and aromatase-transfected human breast

cancer cells – a 10- to 30-fold difference in effectiveness has been found, with letrozole being the more potent. The explanation for the difference in the measurements made in intact cell and cell-free systems is not clear but may be related to uptake of the respective compounds. It might be expected that the intact cell systems would more accurately predict the likely effectiveness of these compounds in the *in vivo* setting.

C. PRECLINICAL MODEL SYSTEMS

Until recently, preclinical modelling of the use of aromatase inhibitors in rodents has been largely limited to premenopausal systems (i.e., those with intact ovarian function) because rodents appear to have little peripheral aromatase activity (in contrast to postmenopausal women, where these drugs have found their greatest application). In these models, the aromatase inhibitors generally have been shown to have good antitumour activity on carcinogen-induced mammary tumours (DeCoster *et al.*, 1992; Brodie *et al.*, 1983). However, the compounds also have had a marked effect on ovarian morphology, with the induction of multiple follicles due to the increase in gonadal stimulation from loss of estrogen feedback on the hypothalamic-pituitary axis (Shetty *et al.*, 1997).

More recently, model systems have focused on the use of aromatase-transfected human MCF7 breast cancer cells in a xenograft model employing athymic nude mice (Lee *et al.*, 1995; Lu *et al.*, 1998). These are more representative of the situation in postmenopausal women and rely on intratumoural aromatase as their primary source of estrogen. Using such models, it has been possible to show the effectiveness of contemporary aromatase inhibitors and compare them with tamoxifen. In general, the inhibitors show greater efficacy than tamoxifen (Lu *et al.*, 1998). As always, the interpretation of these data depends on the comparative pharmacology of the compounds in the mouse and human and the degree to which the experimental tumour represents the range of biological characteristics of human breast cancer.

D. PHARMACOLOGICAL EFFECTIVENESS

1. Peripheral Effects

Two methodologies have been used to estimate the clinical pharmacological effectiveness of aromatase inhibitors. Most studies have accumulated data on the effects of the compounds on plasma estrogen levels. This methodology, however, suffers from a number of deficits. First, it cannot distinguish between effects on production and those that changes in clearance may have. However, a more important issue is the limited sensitivity of plasma estrogen assays: in effect, the maximum degree of suppression that can be shown of primary estrogens (estrone and estradiol) levels using the most sensitive immunoassays available is about

85%. Many assays lack the sensitivity to show even this degree of efficacy. Thus, comparing results between different studies and approaches has little validity.

Of substantially greater value has been application of the more complicated methodology used to measure aromatase activity directly. This involves the injection of [^{3}H]-androstenedione and [^{14}C]-estrone before and during the treatment of women with the respective inhibitor (Jacobs *et al.*, 1991). Collecting urine over a 72-hour period and establishing the [^{3}H]:[^{14}C] ratio in the purified estrogen fractions allow calculation of the peripheral aromatase activity in the patient and the degree of inhibition exerted. An advantage of this methodology is that the inclusion of [^{14}C]-estrone provides an internal standard that permits better comparability of results between studies and over time.

Table I compares the degree of aromatase inhibition achieved among drugs of contemporary importance. This demonstrates that while aminoglutethimide and the second-generation inhibitors suppress aromatase by little more than 90% at their clinically used dosages, the new third-generation compounds approach complete ablation of aromatase activity. In a recent study, letrozole was found to inhibit by greater than 99% in all 12 patients (Geisler *et al.*, 2001).

In contrast to the numerous effects of aminoglutethimide on adrenal steroidal function and other cytochrome P450-dependent processes (e.g., prostaglandin and thyroxine synthesis), the newer aromatase inhibitors essentially are completely specific at clinical dosages. Exemestane causes minor reduction in sex hormone-binding globulin (SHBG) levels, probably due to its androgenic nature. Letrozole has been noted to exert a statistically significant effect on corticotropin (ACTH)-stimulated adrenal function (Bajetta *et al.*, 1999). However, these effects are unlikely to be of clinical significance.

TABLE I

Degree of Whole-body Aromatase Inhibition by Drugs Used in Breast Cancer Clinical Efficacy

Drug	Dose	Mean percentage inhibition	Reference
Aminoglutethimide (+ hydrocortisone)	1000 mg (+ 40 mg)/d	90.6	MacNeill *et al.*, 1992
Formestane	250 mg/2 w (im)	84.8	Jones *et al.*, 1992
Fadrozole	2 mg/d	82.4	Lonning *et al.*, 1991
Vorozole	1 mg/d	93.0	Van der Wall *et al.*, 1993
Letrozole	2.5 mg/d	> 99.1	Geisler *et al.*, 2001
Anastrozole	1 mg/d	97.3	Geisler *et al.*, 2001
Exemestane	25 mg/d	97.9	Geisler *et al.*, 1998

[All analyses, other than Van der Wall *et al.* (1993) were conducted in the Dowsett laboratory.]

2. Premenopausal Women

The observation that formation of multiple ovarian follicles occurs upon administration of aromatase inhibitors in animal model systems has discouraged the widespread investigation of these compounds in premenopausal women (Shetty *et al.*, 1997). Studies with aminoglutethimide generally reveal that estrogen synthesis is largely unaffected by the aromatase inhibitor, although increases in gonadotrophins indicated a degree of compensated inhibition. Application of the more potent steroidal aromatase inhibitor, 4-hydroxyandrostenedione, even at the high dose of 500 mg/week, had no significant impact on premenopausal estrogen levels. The only data from use of a potent nonsteroidal aromatase inhibitor in premenopausal women are from a single-dose study with vorozole. It showed some degree of suppression but duration with repeated doses is unknown. Thus, for the moment, application of aromatase inhibitors to treatment of premenopausal women with breast cancer is limited to their combined usage with gonadotropin-releasing hormone (GnRH) agonists such as goserelin. However, successful use of letrozole in ovulation induction in women with anovulatory infertility recently was reported (Mitwally and Casper, 2001). This is an area that merits substantial further investigation.

3. Intratumoural Effects

The observation that the aromatase enzyme is expressed in both normal and malignant breast has led to substantial investigation of the significance of enzyme source. In postmenopausal women, estrogen that is produced locally likely would cause at least as much stimulation of breast tissues as that by estrogen derived from the circulation. It is clear that aromatase inhibitors effectively suppress estrogen synthesis within the breast in almost all cases (DeJong *et al.*, 1997; Miller, 1999). The degree of residual aromatase activity in tumours is very difficult to estimate, however.

Studies by Miller's group have indicated that a significant association may exist between the presence of intratumoural aromatase and the lesion's response to aromatase inhibitors (Miller and O'Neill, 1987). However, expanding these studies to the large scale required to establish any clinical utility of this observation has been limited by the relatively large mass of fresh tissue required to make the measurements. It is hoped that, in the near future, sensitive and specific antibodies will permit the characterisation of aromatase expression in breast carcinomas using immunohistochemical techniques. This will allow the relationship between aromatase and the clinical benefit derived from aromatase inhibitors to be established more conclusively.

4. Application of Aromatase Inhibitors in Breast Cancer

Most data on the effectiveness of aromatase inhibitors in breast cancer are from studies of locally advanced or metastatic disease. Comparative clinical trials have established that the third-generation compounds letrozole and vorozole are more effective than aminoglutethimide and that letrozole is more effective than fadrozole (Bergh *et al.*, 1997; Gershanovich *et al.*, 1998; Tominaga *et al.*, 1998). These data are important because they establish that drugs that achieve essentially complete inhibition of aromatase are more efficacious than those that suppress aromatase by approximately 90%. Thus, over this range of suppression, a clinical response:dose relationship with aromatase inhibition seems to exist.

All of the aromatase inhibitors have shown some benefit over megestrol acetate, the previously standard second-line agent for advanced breast cancer treatment (Buzdar *et al.*, 1996; Dombernowsky *et al.*, 1998; Kaufmann *et al.*, 2000). Aromatase inhibitors have supplanted this agent as the second-line agent of choice.

The most important clinical data, some of the most important in the hormonal treatment of breast cancer for the last decade, have matured over the last couple of years. For over 20 years, tamoxifen was the most widely used agent in breast cancer treatment because of its efficacy and excellent tolerability. Comparative studies of tamoxifen with aminoglutethimide and the second-generation inhibitors, 4-hydroxyandrostenedione and fadrozole, showed no improvement in efficacy of these inhibitors over tamoxifen (Perez Carrion *et al.*, 1994; Thurlimann *et al.*, 1996) in advanced beast cancer. However, large clinical trials of tamoxifen versus the third-generation nonsteroidal aromatase inhibitors, anastrozole and letrozole, have reported greater efficacy of the aromatase inhibitors over the antiestrogen (Nabholtz *et al.*, 1999; Bonneterre *et al.*, 2000; Mouridsen *et al.*, 2001). Thus, in patients with estrogen receptor- and/or progesterone receptor-positive advanced breast cancer, an aromatase inhibitor will now be the first agent of choice.

5. Neoadjuvant Therapy

The conventional approach to the management of operable breast cancer is the immediate removal of the primary lesion and the subsequent delivery of adjuvant medical treatment. Neoadjuvant therapy reverses this process such that medical treatment is given prior to surgery. A series of small studies suggested that aromatase inhibitors might be more effective than tamoxifen in this context (Dixon *et al.*, 1999), although this was not confirmed in a relatively small study of vorozole versus tamoxifen (Harper-Wynne *et al.*, 2001). Most importantly, however, a large, double-blind, randomized study has confirmed that letrozole is a substantially more effective agent in primary breast cancer therapy than is tamoxifen, thus confirming the enhanced effectiveness of this compound in

advanced disease (Ellis *et al.*, 2001). Neoadjuvant studies have the advantage of allowing correlation between the presence of specific biomarkers in the disease before treatment and the clinical response in the same lesion immediately afterwards. Particularly provocative data (Ellis *et al.*, 2001) indicate that letrozole is more effective in steroid-receptor positive tumours, which are also either epidermal growth factor (EGF) receptor or HER-2 positive, than in those that are growth factor receptor negative (Figure 4). While this observation concurs with some preclinical studies (Kurokawa *et al.*, 2000), further clinical evidence is required to confirm these potentially highly important data.

6. Adjuvant Therapy

Survival benefit rarely is shown in comparative advanced breast cancer studies. Medical agents generally need to be applied immediately after surgery as adjuvant therapy to achieve marked improvements in survival. It is well established that in estrogen receptor-positive disease, tamoxifen reduces the odds of death by about 30% after 5 years of use (Early Breast Cancer Trialists'
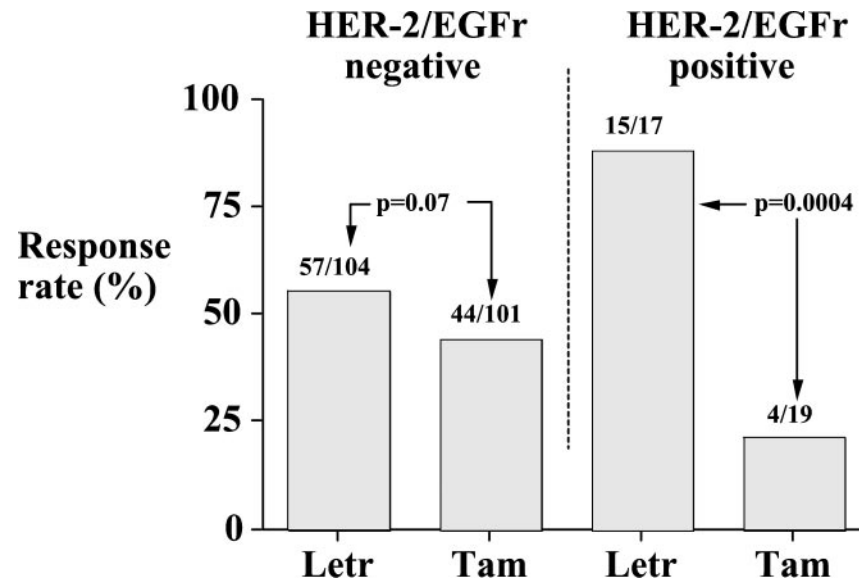

FIG. 4. Response rates to letrozole and tamoxifen according to HER2/EGFr studies in a neoadjuvant clinical trial in primary breast cancer. All patients were either EDR or PgR positive. [Data from Ellis MJ, Coop A, Singh B, Mauriac L, Llombert-Cussac A, Janicke F, Miller WR, Evans DB, Dugan M, Brady C, Quebe-Fehling E, Borgs M 2001 Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/or ErbB-2-positive, estrogen receptor-positive primary breast cancer: evidence from a phase III randomized trial. J Clin Oncol 19:3808–3816.]

Collaborative Group, 1998). The very encouraging data from comparisons between tamoxifen and aromatase inhibitors in advanced breast cancer and neoadjuvant therapy prompt the hope that the inhibitors can improve upon the survival seen with tamoxifen in adjuvant therapy. Thus, several very large comparative trials of aromatase inhibitors versus tamoxifen and others will assess the application of these agents in sequence in patients with no apparent metastatic disease. It is important to note that long-term side effects may be as important as efficacy data in this population of patients. It is clear that estrogen deprivation might have adverse effects on the integrity of bone in postmenopausal women (Harper-Wynne *et al.*, 2001). Possible effects on cardiovascular and cognitive function could adversely influence the applicability of inhibitors in this context. Thus, each of the ongoing, large studies is systematically collecting detailed information on these issues of tolerability.

7. Prospects for Preventative Use

The involvement of estrogens as promoters of breast cancer is very well established, being based on a wealth of indirect epidemiological evidence (Henderson, 1989). For example, early menarche and late menopause, which extend the exposure of the breast to cyclical estrogenic stimuli, both increase risk of breast cancer. Obesity in postmenopausal women, which increases the plasma concentrations of estrogens, and the application of hormone replacement therapy also enhance breast cancer risk. Of particular note is a series of prospective studies that have collected plasma from women many years before they developed breast cancer. This study has consistently indicated that increased plasma levels of estrogen are associated with increased breast cancer incidence (Thomas *et al.*, 1997). These observations have encouraged the experimental application of agents that can attenuate the estrogenic stimulation of the breast as agents for the prophylaxis of breast cancer. The observations that tamoxifen can reduce the incidence of breast cancer in women at increased risk by approximately 50% over a 4-year period (Fisher *et al.*, 1998; Cauley *et al.*, 2001) and that the selective estrogen receptor modulator (SERM) raloxifene reduces the incidence of breast cancer in women at high osteoporotic risk have both provided support to this concept and have led aromatase inhibitors being considered as potential preventative agents. The observation that fadrozole can markedly reduce the incidence of sporadic mammary tumours in Sprague-Dawley rats over their lifetime (Gunson *et al.*, 1995) provides compelling support for this concept.

If the effects of tamoxifen and raloxifene are dependent on their antagonising estrogen signalling, the aromatase inhibitors would be expected to be at least as effective in preventing breast cancer. An additional advantage of the inhibitors over the SERMs is that the aromatase inhibitors would be expected to reduce development of genotoxic DNA adducts that may result from the chemical

reactivity of catecholestrogen metabolites and have a theoretical basis for being involved with breast cancer development (Cavalieri *et al.*, 1997). However, in the chemopreventive setting, side effects are of particular importance, especially in women at only moderate risk. SERMs such as tamoxifen and raloxifene may have an advantage over aromatase inhibitors in this regard. The former have been found to reduce the incidence of osteoporotic fractures (Fisher *et al.*, 1998; Ettinger *et al.*, 1999) and to have effects on lipids that might be expected to have a beneficial effect on cardiac disease (Powles *et al.*, 1989; Johnston *et al.*, 2000). In contrast, the SERMs have deleterious effects on incidence of thrombo-embolic events and tamoxifen enhances the incidence of endometrial cancer (Fisher *et al.*, 1998). Any studies of aromatase inhibitors in the chemopreventative setting will need to consider these issues and compare the effectiveness and side effects of aromatase inhibitors with those of tamoxifen.

One approach to reducing the possible impact of aromatase inhibitors on normal tissues would be to target the therapy at those women with the highest exposure to endogenous estrogens. These could, for example, be identified by plasma estrogen measurements or, alternatively, by assessment of bone mineral density (BMD). BMD tends to reflect long-term estrogen exposure and is itself linked to breast cancer incidence. Rather than obliterating the residual estrogen in these postmenopausal women, a partial reduction in estrogen levels to those in women with low risk of breast cancer is an attractive concept. However, this may be difficult to achieve with low-dose aromatase inhibitors because of variable intersubject pharmacokinetics and pharmacodynamics.

IV. Selective Aromatase Modulators

Another approach to reducing the risk of side effects is to develop tissue-specific inhibitors of aromatase expression, rather than inhibitors of the catalytic activity. Third-generation aromatase inhibitors are finding utility in the treatment of estrogen-dependent diseases such as breast cancer and, more recently, endometriosis (Zeitoun *et al.*, 1999). However, a disadvantage is that they inhibit aromatase activity in a global fashion and thus could adversely impact sites where estrogen is required for normal function (e.g., maintenance of bone mineralization, prevention of hepatic steatosis and loss of cognitive function). The concept of selective aromatase modulators (SAMs) is made possible by three considerations. The first is that, in postmenopausal women and in men, estrogen largely acts at a local level in sites where it is produced in a paracrine or even intracrine fashion. Second, aromatase expression in these different tissue sites of expression is regulated by tissue-specific promoters. Third, various tissue-specific aromatase promoters employ different signalling pathways and thus different cohorts of transcription factors. Therefore, it is possible to envision

tissue-specific inhibition of aromatase expression in a similar fashion to the concept of tissue-specific regulation of estrogen action (the concept of SERMs).

As indicated previously, when a breast tumour is present, aromatase activity within the tumour and its surrounding adipose tissue is such that intratumoural estradiol levels are at least an order of magnitude greater than those in circulating plasma of postmenopausal women. (This may be one reason why HRT carries relatively little increased risk of breast cancer.) This is because the tumour produces factors that stimulate aromatase expression locally. This stimulation is associated with switching of the aromatase gene promoter from I.4 to promoter II, the gonadal-type promoter (Harada *et al.*, 1993; Agarwal *et al.*, 1996; Zhou *et al.*, 1997) (Figure 5). Thus, drugs that target promoter II-driven expression of aromatase would be most useful. In postmenopausal women, this promoter would appear to be exclusively utilized in tumour-containing breast tissue (and
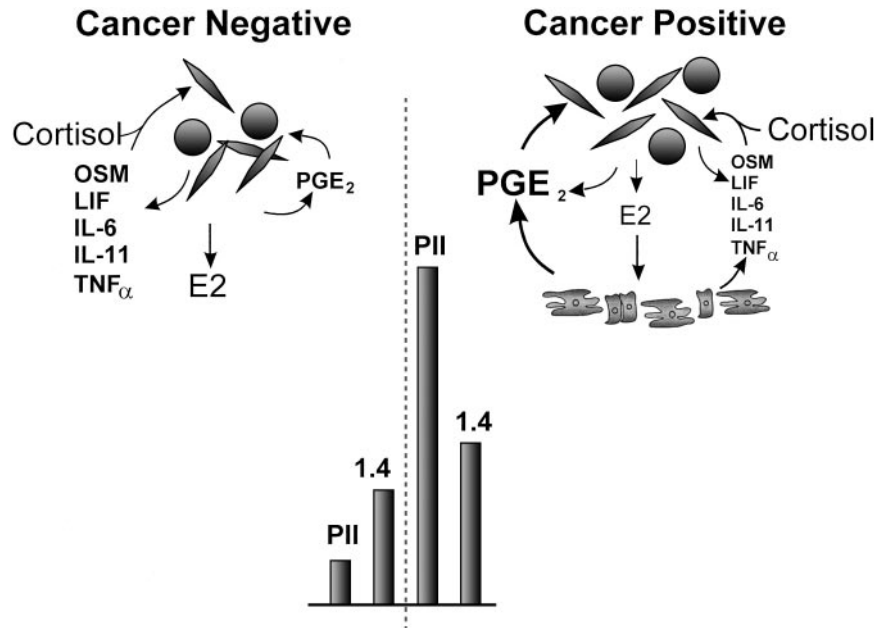

FIG. 5. Proposed regulation of aromatase gene expression in breast adipose tissue from cancer-free individuals and from those with breast cancer. In the former case, expression is stimulated primarily by class I cytokines and tumour necrosis factor alpha produced locally, in the presence of systemic glucocorticoids. As a consequence, promoter I.4-specific transcripts of aromatase predominate. In the latter case, aromatase expression is increased and promoter II-specific transcripts of aromatase predominate, suggesting a major role for prostaglandin E2 (PGE_2) in aromatase expression. PGE_2 in this case could be derived from the tumourous epithelium, tumour-derived fibroblasts, and/or macrophages recruited to the tumour site.

in endometriotic plaques) (Agarwal *et al.*, 1996; Zeitoun *et al.*, 1999). Thus, bone in particular, which does not express promoter-II specific transcripts (Shozu and Simpson, 1998), would be spared.

Tumour-derived factors include prostaglandin E (PGE)$_2$ (Schrey and Patel, 1995), which stimulates adenylate cyclase in adipose stromal cells, and promoter II, which is regulated by cAMP. It was found that PGE$_2$ is a powerful stimulator of aromatase expression in these cells via promoter II (Zhao *et al.*, 1996). Moreover, expression of the CYP19 gene was correlated with COX-1 and COX-2 expression in human breast cancer and normal tissue specimens (Brueggemeier *et al.*, 1999). A case-control study published some years ago indicated that daily use of nonsteroidal, anti-inflammatory drugs such as ibuprofen reduced the incidence of breast tumours by up to 40% (Harris *et al.*, 1996). More recently, it has been shown that the COX-2 inhibitor, celecoxib, has strong chemopreventive activity against mammary carcinoma in rats (Harris *et al.*, 2000). From these considerations, it appears likely that inhibition of aromatase expression selectively in breast tissue could play an important role in this chemopreventive action of cyclo-oxygenase inhibitors, which therefore might qualify as the first generation of SAMs. However, ongoing research on regulation of aromatase expression in the breast is likely to uncover other candidates in the not-too-distant future.

ACKNOWLEDGMENTS

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