Use of aromatase inhibitors in breast carcinoma

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Abstract

Aromatase, a cytochrome P-450 enzyme that catalyzes the conversion of androgens to estrogens, is the major mechanism of estrogen synthesis in the post-menopausal woman. We review some of the recent scientific advances which shed light on the biologic significance, physiology, expression and regulation of aromatase in breast tissue. Inhibition of aromatase, the terminal step in estrogen biosynthesis, provides a way of treating hormone-dependent breast cancer in older patients. Aminoglutethimide was the first widely used aromatase inhibitor but had several clinical drawbacks. Newer agents are considerably more selective, more potent, less toxic and easier to use in the clinical setting. This article reviews the clinical data supporting the use of the potent, oral competitive aromatase inhibitors anastrozole, letrozole and vorozole and the irreversible inhibitors 4-OH andro-stenedione and exemestane. The more potent compounds inhibit both peripheral and intra-tumoral aromatase. We discuss the evidence supporting the notion that aromatase inhibitors lack cross-resistance with antiestrogens and suggest that the newer, more potent compounds may have a particular application in breast cancer treatment in a setting of adaptive hypersensitivity to estrogens. Currently available aromatase inhibitors are safe and effective in the management of hormone-dependent breast cancer in post-menopausal women failing antiestrogen therapy and should now be used before progestational agents. There is abundant evidence to support testing these compounds as first-line hormonal therapy for metastatic breast cancer as well as part of adjuvant regimens in older patients and quite possibly in chemoprevention trials of breast cancer.

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Introduction

Epithelial cells of the normal breast undergo dramatic changes during various events in a woman's life such as puberty, the follicular and luteal phases of the menstrual cycle, pregnancy and menopause. The co-ordinated interaction of growth factors and steroid hormones regulate the proliferation and differentiated function of epithelial and stromal cells in the normal mammary gland. The key growth factors are insulin-like growth factor-I, prolactin, insulin, the fibroblast growth factor family of growth factors and growth hormone, and major steroid hormones are estradiol, progesterone and testosterone (Frantz & Wilson 1998).

For the process of inducing breast cancer, estrogens appear to play a predominant role. These sex steroids are believed to initiate and promote the process of breast carcinogenesis by enhancing the rate of cell division and reducing time available for DNA repair. An emerging new concept is that estrogens can be metabolized to catecholestrogens and then to quinones which directly damage DNA. These two processes - the estrogen receptor-mediated, genomic effects on proliferation and the receptor-independent, genotoxic effects of estrogen metabolites - can act either in an additive or synergistic fashion to cause breast cancer (Santen *et al*. 1999).

Breast cancers which arise in patients can be divided into two subtypes: those which are dependent upon hormones for growth and those which grow independently of hormonal stimulation (Santen *et al*. 1990). In the hormone-dependent subtype, the role of estrogens as modulators of mitogenesis overrides the influence of other factors. These sex steroids stimulate cell proliferation directly by increasing the rate of transcription of early response genes such as c-myc and indirectly through stimulation of growth factors which are produced largely in response to estrogenic regulation (Dickson & Lippman 1995).

Based upon the concept that estrogen is the proximate regulator of cell proliferation, two general strategies were developed for treatment of hormone-dependent breast cancer: blockade of estrogen receptor action and inhibition of estradiol biosynthesis. Antiestrogens such as tamoxifen bind to the estrogen receptor and interfere with transcription of estrogen-induced genes involved in regulating cell proliferation. Clinical trials showed tamoxifen to be effective in inducing objective tumor regressions and to be associated with minimal side-effects and toxicity. The second strategy, blockade of estradiol biosynthesis, was demonstrated to be feasible using the steroidogenesis inhibitor, aminoglutethimide, which produced tumor regressions equivalent to those observed with tamoxifen (Santen *et al*. 1990). However, side-effects from aminoglutethimide were considerable and its effects on several steroidogenic enzymes required concomitant use of a glucocorticoid (Santen *et al*. 1982). Consequently, tamoxifen became the preferred, first-line endocrine agent with which to treat advanced breast cancer. However, the clinical efficacy of aminoglutethimde focused attention upon the need to develop more potent, better tolerated, and more specific inhibitors of estrogen biosynthesis.

Inhibition of estradiol biosynthesis

Multiple strategies could be used to inhibit estradiol biosynthesis as a treatment for estrogen-dependent breast cancer. Inhibition of several enzymes in the steroidogenic pathway, including cholesterol side-chain cleavage, 3 beta-ol-dehydrogenase-delta 4-5 isomerase, 17-alpha hydroxylase, 17-beta hydroxysteroid dehydrogenase, estrone sulfatase, and aromatase, could be used to reduce the biosynthesis of estradiol and potentially cause hormone-dependent breast tumor regression. An additional strategy is the use of exogenous glucocorticoid to inhibit release of adrenocorticotropin (ACTH) and suppress estrogen production. Finally, synthetic progestins such as megestrol acetate and medroxy-progesterone acetate exert glucocorticoid effects and inhibit estradiol synthesis by suppressing ACTH.

The ideal strategy would be to block the synthesis of estrogen without inhibiting production of other important steroids or giving pharmacological amounts of progestins or glucocorticoids. For this reason, blockade of the terminal step in estradiol biosynthesis catalyzed by the enzyme aromatase is considered a more specific and therefore preferable strategy. Several pharmaceutical companies sought to develop potent aromatase inhibitors designed to specifically block estrogen biosynthesis without altering glucocorticoid and mineralocorticoid synthesis, and without requiring addition of large amounts of progestins or exogenous glucocorticoid.

Physiology and regulation of aromatase

Aromatase is a cytochrome P-450 enzyme which catalyzes the rate-limiting step in estrogen biosynthesis, the conversion of androgens to estrogens (Simpson *et al*. 1997, Sasano & Harada 1998). Two major androgens, androstenedione and testosterone, serve as substrates for aromatase. The aromatase enzyme consists of a complex containing a cytochrome P-450 protein as well as the flavoprotein NADPH cytochrome P-450 reductase (Simpson *et al*. 1997). The gene coding for the cytochrome P-450 protein (P-450 AROM) exceeds 70 kb and is the largest of the cytochrome P-450 family (Simpson *et al*. 1993). The cDNA of the aromatase gene contains 3.4 kb and encodes a polypeptide of 503 amino acids with a molecular weight of 55 kDa. Approximately 30% homology exists with other cytochrome P-450 proteins. Because its overall homology to other members of the P-450 superfamily is low, aromatase belongs to a separate gene family designated CYP19.

Recent studies indicate that the transcription of the aromatase gene is highly regulated (Simpson *et al*. 1989, 1993, 1997). The first exon of the aromatase gene is transcribed into aromatase message but not translated into protein. There exist nine alternative first exons which can initiate the transcription of aromatase. Each of these alternate exons contains upstream DNA sequences which can either enhance or silence the transcription of aromatase. Different tissues utilize specific alternate exons to initiate transcription. For example, the placenta utilizes alternate exon I.1, the testis alternate exon II, adipose tissue I.3 and I.4 and brain If. Enhancers which react with upstream elements of these alternate exons markedly stimulate the rate of transcription of the aromatase gene. Thus, each tissue can regulate the amount of aromatase transcribed in a highly specific manner (Simpson *et al*. 1993).

Aromatase expression occurs in many organs, including ovary, placenta, hypothalamus, liver, muscle, adipose tissue, and breast cancer itself. Aromatase catalyzes three separate steroid hydroxylations which are involved in the conversion of androstenedione to estrone or testosterone to estradiol. The first two give rise to 19-hydroxy and 19-aldehyde structures and the third, although still controversial, probably also involves the C-19 methyl group with release of formic acid (Fishman & Hahn 1987). This enzymatic action results in the saturation of the A-ring of the steroid molecule to produce an aromatic structure, hence the term aromatization.

In the premenopausal state, the major source of aromatase and of its substrates is the ovary. However, extra-glandular aromatization of adrenal substrates in peripheral sites such as fat, liver and muscle also contributes substantially to the estrogen pool in the early
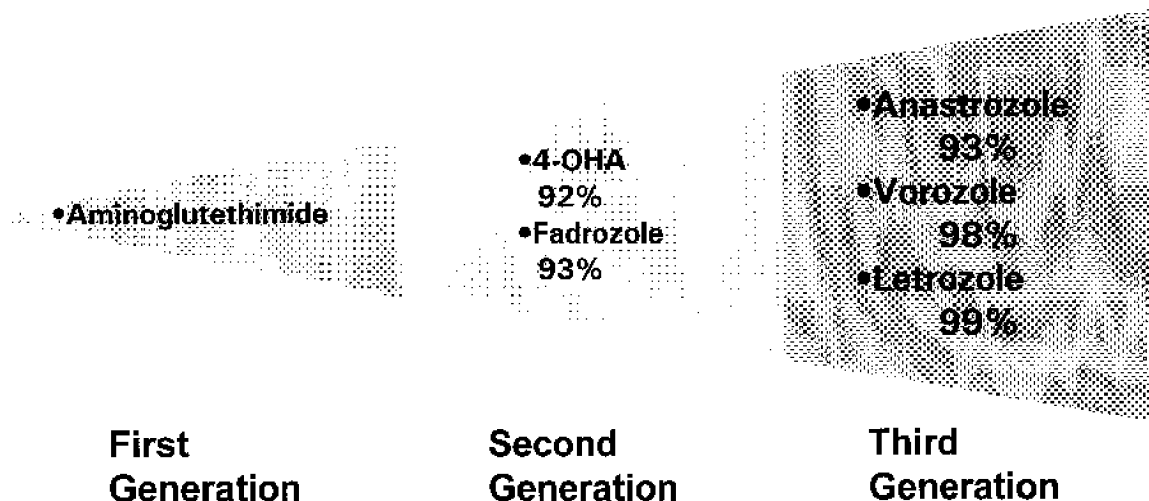
AROMATASE INHIBITORS
Potency

Figure 1 Diagrammatic representation of the potency of aromatase inhibitors as reflected by the isotopic kinetic method for determining degree of aromatase inhibition. The percent conversion of androstenedione to estrone is measured isotopically, correcting for losses of estrone by giving ^{14}[C] estrone tracer. Values indicated represent percent inhibition of total body aromatase.

follicular and late luteal phases of the menstrual cycle. In the postmenopausal state, the ovary loses its complement of aromatase enzyme although it does continue to secrete androstenedione. The adrenal subsumes the primary role of providing substrate for aromatase by directly secreting testosterone and androstenedione. In addition, dehydro-epiandrosterone and its sulfate are secreted by the adrenal and converted into the aromatase substrates, andros-tenedione and testosterone, in peripheral tissues. The major source of the aromatase enzyme in postmenopausal women is peripheral tissues and particularly fat and muscle.

Recent studies identified an additional, important site of estrogen production, breast tissue itself. Two-thirds of breast carcinomas contain aromatase and synthesize biologically significant amounts of estrogen locally in the tumor (Abul-Hajj *et al*. 1979, Miller & O'Neil 1987, Santen *et al*. 1994). Proof of local estradiol synthesis includes measurement of tumor aromatase activity by radiometric or product isolation assays, by immuno-histochemistry, by demonstration of aromatase mRNA in tissue, and by aromatase enzyme assays performed on cells isolated from human tumors and grown in cell culture. The expression of aromatase is highest in the stromal compartment of breast tumors (Santen *et al*. 1994) but is present in epithelial cells as well. In breast tissue

surrounding the tumors, preadipocyte fibroblasts contain aromatase activity that can be detected by biochemical assay or immunohistochemical staining (Miller & O'Neil 1987, Santen *et al*. 1994). Normal breast tissue also contains aromatase as documented by immunohisto-chemistry, by demonstration of aromatase message, and by enzyme assays of cultured cells (Mor *et al*. 1998, Brodie *et al*. 1999).

The biologic relevance of *in situ* estrogen production by aromatase has been demonstrated by xenograft experiments which compare tumors containing and not containing aromatase (Yue *et al*. 1998). Human breast cancer cells transfected permanently with the aromatase enzyme are compared with cells transfected with irrelevant DNA. In these experiments, tumors containing the transfected aromatase enzyme have higher amounts of estrogen and grow faster than those with transfection of irrelevant DNA. Further, these experiments show that local production of estradiol in the tumor is a greater source of estrogen than uptake from plasma (Yue *et al*. 1998). Taken together, these studies support the importance of *in situ* estrogen production by breast tumors and suggest that aromatase inhibitors in patients must be sufficiently potent to block intra-tumoral aromatase.

Breast tumor tissue aromatase can be regulated by several enhancers of aromatase transcription (Simpson *et*

AROMATASE INHIBITORS
Spectrum of Action

Aminoglutethimide

•Induction
  hepatic enzymes

•Inhibition
  cortisol
  aldosterone
  thyroxine
  aromatase

4-OHA
•Inhibition
  aromatase
Fadrozole
•Inhibition
  aromatase
  aldosterone

•Inhibition
aromatase only

First Generation   **Second Generation**   **Third Generation**
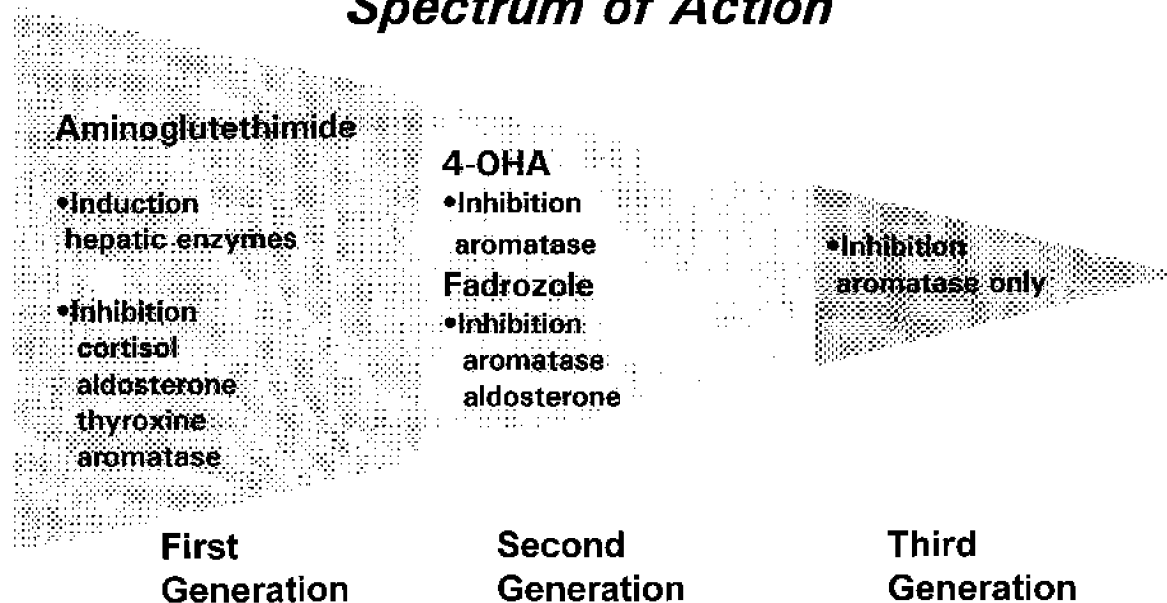
Figure 2 Diagrammatic representation of the spectrum of action of first through third generation aromatase inhibitors. With development of newer inhibitors, the spectrum of action narrows. The third generation aromatase inhibitors act exclusively on the aromatase enzyme and do not appear to exert additional effects.

al. 1997). Dexamethasone, phorbol esters, cyclic AMP, interleukin 6, and prostaglandins can all stimulate aromatase transcription in cultured breast cancer cells and specifically in the stromal components. Interestingly, products secreted by epithelial cells in the breast tumors appear to stimulate aromatase in the stroma and provide a means for autoregulation of tumor growth through estrogen production. A rather novel means of regulation of aromatase levels was also recently described - the stabilization of degradation of enzyme (Harada *et al.* 1999). Aromatase inhibitors bind to the active site of the enzyme and, through mechanisms not completely understood, prevent proteolysis of the aromatase protein. Each of these mechanisms may enhance the amount of aromatase in tumor tissue and increase the need for very potent aromatase inhibitors.

Development of aromatase inhibitors

The first aromatase inhibitors were discovered nearly 30 years ago and included aminoglutethimide and testolo-lactone (Santen *et al.* 1990). Testolactone was not very potent as an inhibitor, and aminoglutethimide blocked several P-450-mediated enzymatic reactions and was

associated with troublesome side-effects. On the other hand, aminoglutethimide appeared to be quite effective in causing tumor regressions in patients with breast cancer. For this reason, pharmaceutical companies and individual investigators focused upon developing more potent and specific inhibitors. Second and third generation inhibitors were developed with 10- to 10000-fold greater potency than aminoglutethimide and greater specificity (Figs 1 and 2). The half-lives of the inhibitors increased with synthesis of more potent inhibitors. The third generation aromatase inhibitors are capable of decreasing the levels of circulating estrogens to a greater extent than the first and second generation inhibitors in postmenopausal women with hormone-dependent breast cancer. Hypothetically, these highly potent agents could also reduce levels of intra-tumoral aromatase activity to a greater extent than the earlier inhibitors but this has not yet been examined.

Pharmacologic classification of aromatase inhibitors

A convenient classification divides inhibitors into mechanism based or 'suicide inhibitors' (Type 1) and competitive inhibitors (Type II) (Brodie 1993). Suicide
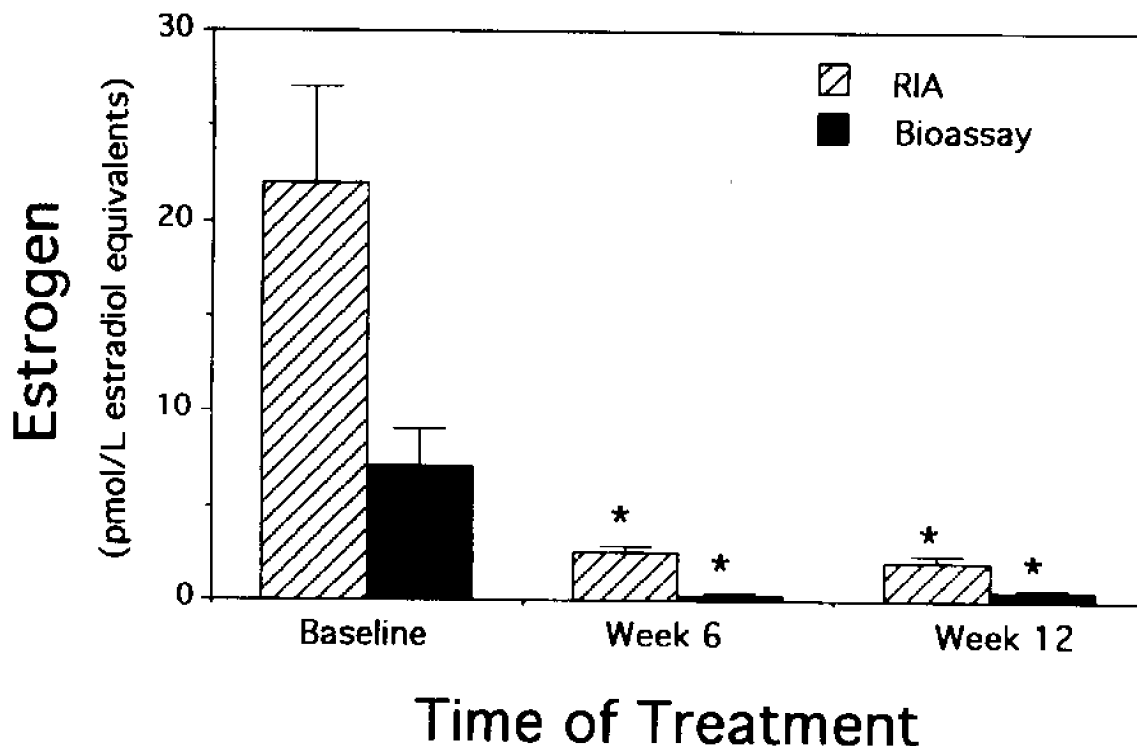
Figure 3 Inhibition of plasma estrogen levels as assessed by RIA and by an ultrasensitive, recombinant DNA-based bioassay (Jones *et al.* 1992). Basal estradiol levels are approximately threefold lower when measured by the ultrasensitive assay. During administration of the aromatase inhibitor, levels fall to 0.05-0.07 pmol/l as assessed by the ultrasensitive assay and to 2-5 pg/ml with the standard RIA.
*$P < 0.01$ vs baseline.

inhibitors initially compete with natural substrates (i.e. androstenedione and testosterone) for binding to the active site of the enzyme. The enzyme, then, specifically acts upon the inhibitor to yield reactive alkylating species which form covalent bonds at or near the active site of the enzyme. Through this mechanism, the enzyme is irreversibly inactivated. Competitive inhibitors, on the other hand, bind reversibly to the active site of the enzyme and prevent product formation only as long as the inhibitor occupies the catalytic site. Whereas mechanism-based inhibitors are exclusively steroidal in type, competitive inhibitors consist both of steroidal and non-steroidal compounds (Brodie 1993).

Methods used to demonstrate aromatase inhibition

The standard method to study aromatase inhibitors in patients is to measure either plasma or urinary estrogen by RIA. Early studies demonstrated 50-80% inhibition of plasma or urinary estrone or estradiol (Santen *et al.* 1978,

1981, 1982). Another method involved measurement of each estrogen metabolite in urine with calculation of total aromatized product. This technique provided results similar to those from measurements of urinary estrone or estradiol (Lipton *et al.* 1995). Using these plasma or urinary methods, each agent appeared to suppress estrogen levels to concentrations approaching the sensitivity of the RIAs used. To gain greater specificity and sensitivity, investigators utilized the isotopic kinetic technique of Siiteri *et al.* to measure total body aromatase (Grodin *et al.* 1973, Santen *et al.* 1978, Jones *et al.* 1992, Dowsett *et al.* 1995). This required administration of tritiated androstenedione and [14][C]-estrone to patients under steady-state conditions and measurement of radiochemically pure tritiated estrone and estradiol (Santen *et al.* 1978). The [14][C]-estrone allowed correction for losses during multiple purification steps. Using this technique, the degree of inhibition with various inhibitors ranged from 90 to 99%.

From these observations, it was recognized that more sensitive plasma assays of estradiol were needed. One

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