Aromatase and Aromatase Inhibitors in Breast Cancer Treatment

Prafulla M Sabale1*, Vidya P. Sabale2, Lata C. Potey1

1Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur-440033 M.S.

2Dadasaheb Balpande College of Pharmacy, Besa, Nagpur, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur-440033 M.S

Received 11 November 2018; received in revised form 26 December 2018; accepted 26 December 2018

*Corresponding author E-mail address: prafullasable@yahoo.com

ABSTRACT
Breast cancer is the most common cancer diagnosed in women, remains the second most common causes of death in women in the Western world. With advances in screening, diagnosis and treatment, the death rate for breast cancer patients has declined. Estrogens are responsible for the growth of hormone-dependant breast cancer. Regulation of estrogen biosynthesis is considered as a potential therapeutic strategy for the control of breast cancer. The enzyme aromatase catalyzes conversion of androgens into estrogens in the last step of estrogen biosynthesis. Inhibition of aromatase is adopted as an efficient approach for the prevention and treatment of breast cancer. Many steroidal and non steroidal aromatase inhibitors have been developed and are used clinically for breast cancer therapy. At present, two types of aromatase inhibitors, irreversible steroidal inhibitor and reversible nonsteroidal inhibitors are used in the treatment. Mostly, third-generation aromatase inhibitors are used as potent inhibitors of the aromatase enzyme, which catalyzes the conversion of androgen to estrogen which is the last step in estrogen biosynthesis. This review article aims to highlight about aromatase enzyme, its mechanism and aromatase inhibitors used in the treatment of breast cancer.

KEYWORDS
Breast Cancer; Aromatase enzyme; Aromatase Inhibitors; Exemestane; Anastrazole; Letrozole.
1. INTRODUCTION

Breast cancer is a malignant (cancerous) growth originating from breast tissue, most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk, commonly known as ductal carcinomas and lobular carcinomas. Less common are inflammatory breast cancer and Paget’s disease of the nipple. There are many different types of breast cancers, with different stages (spread), aggressiveness and genetic makeup, survival varies greatly depending on many factors.\(^1\)

Breast cancer is the most common cancer diagnosed in women, and despite advances in treatment, remains the second most common causes of death in women in the Western world. Generally, it is thought of as a disease of older women; however 22% of cases occurs in those below the age of 50. Worldwide, more than one million women developed breast cancer yearly and near about half of these cases has been diagnosed in the Unites States and Europe. Moreover, nearly 40% of the women have been died due to breast cancer. Approximately two-thirds of postmenopausal breast cancer patients have estrogen-dependent breast cancer, which contains estrogen receptors and requires estrogen for tumor growth. In 2013, breast cancer caused 464,000 deaths worldwide (7% of cancer deaths).\(^2\) Heightened awareness of breast cancer risks in the past decades have led to an increase in the number of women undergoing mammography for screening, leading to detection of cancers in earlier stages and a resultant improvement in survival rates. Still, breast cancer is the most common cause of death in women between 45-55 years of age.\(^3\)

With advances in screening, diagnosis and treatment, the death rate for breast cancer patients has declined. In fact, about 90% of women newly diagnosed with breast cancer can survive for at least five years. Research is continuously going on to develop even more effective screening and treatment programs.\(^4\) Prolonged exposure to circulating estrogens contributes to increased incidences of breast cancer. Removal of estrogens via oophorectomy decreases the risk of breast carcinoma. In postmenopausal women, large part of estrogens in the breast tissue are derived from \textit{in situ} biosynthesis using androgens as a substrate. Major sites of estrogen production are ovaries in premenopausal women, placenta in pregnant women and peripheral tissues such as fat, muscles and breast tissues in postmenopausal women.\(^5\)

1.1 Estrogens

Estrogens are essential regulators of many physiological processes including maintenance of the female sexual organs, the reproductive cycle and numerous neuroendocrine functions. In breast cancer, through binding to their target receptor, they promote proliferation of breast cancer cells.\(^6\) Estrogens enhance the growth and proliferation of certain target cells, such as breast epithelial cells and estrogen-dependent mammary carcinoma cells.\(^7\) The binding of estrogens to receptor is the initial step in estrogen-mediated stimulation of DNA synthesis and cell replication in target tissue.\(^8,9,10,11\)

1.2 Aromatase Enzyme

First time the Aromatase enzyme had been isolated by Ryan in 1959 from the microsomal function of fresh human placental tissue.\(^12\) Aromatase is from the family of cytochrome P450 enzyme that converts androgens including androstenedione and testosterone to the estrogen products, estrone and estradiol, respectively.\(^13\) This enzyme plays a key role in the regulation of
these sex steroids. The aromatase gene, designated as CYP19, encodes the cytochrome P450_{arom}
and consists of 10 exons, with the exact size of the gene exceeding 70 kilobases. The gene is
located on chromosome 15q21.1. The full length DNA of 3.4 kilobases encodes for a protein of
503 amino acids with a molecular weight of approximately 55,000 daltons.\textsuperscript{14} The amino acid
sequence of P450_{arom} is distinct from other members of the P450 cytochrome family and drugs
have been developed with selectivity towards the cytochrome P450 moiety in aromatase,
permitting more specific inhibition.

The enzyme complex is bound in the endoplasmic reticulum of the cell and is comprised of two
major proteins\textsuperscript{15, 16} One protein is cytochrome P450_{arom}, a heme protein that converts C19
steroids (androgens) into C18 steroids (estrogens) containing a phenolic A ring. The second
protein is NADPH-cytochrome P450 reductase, which transfers reducing equivalents to
cytochrome P450_{arom}. Three moles of NADPH and three moles of oxygen are used in the
conversion of one mole of substrate into one mole of estrogen product.\textsuperscript{17,18}

1.3 Mechanism of Aromatization
Aromatization of androstenedione proceeds via three successive oxidation steps, in which first
two steps includes hydroxylation of the angular C-19 methyl group (Figure 1) to affords 19-
hydroxy intermediate, reaction occurs with classical mechanism of P450 enzymes.\textsuperscript{19, 20} The second
hydroxylation reaction stereo selectively removes the 19-pro-R hydrogen without an
apparent kinetic isotope effect to yield the 19-gem-diol, which may dehydrate to the 19-
aldehyde.\textsuperscript{21} The third step oxidatively cleaves the C10-C19 bond, resulting in aromatization of
the steroid A-ring and release of formic acid. A number of mechanisms have been proposed for
the aromatization process. One of the mechanisms which received significant favor involves
nucleophilic attack of the 19-aldehyde by the reduced ferrous dioxygen intermediate. The resulting peroxy hemiacetal is suggested to decay via a process by which the proximal oxygen atom removes the 1β-hydrogen, resulting in aromatization of the steroid A ring and releases the
formic acid.\textsuperscript{22}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{reaction_mechanism}
\caption{Reaction mechanism for estrogen biosynthesis by aromatase}
\end{figure}
1.4 Structure of Aromatase

Ghosh et al. identified the crystal structure of human placental aromatase enzyme having androstenedione as a co-crystallized ligand and deposited in protein data bank with ID 3EQM (Figure 2). The residues comprising the catalytic cleft of aromatase enzyme are Ile 305, Ala 306, Asp 309 and Thr 310 from the I-helix, Phe 221 and Trp 224 from the F-helix, Ile 133 and Phe 134 from the B-C loop, Val 370, Leu 372 and Val 373 from the K-helix–b3 loop, Met 374 from b3 and Leu 477 and Ser 478 from the b8–b9 loop (Figure 2).

The 17-keto oxygen of the substrate makes a hydrogen bond (2.8 Å) with the backbone amide of Met 374 and a weak contact (3.4 Å) with NH1 of Arg 115 (Figure 3). The 3-keto oxygen is 2.6 Å from the carboxylate Oδ2 of the Asp 309 side chain indicating that the carboxylate moiety may be protonated.

Figure 2: A ribbon diagram showing the overall structure. The N terminus, starting at residue 45 is coloured dark blue and the C terminus ending at residue 496 is coloured red. The α-helices are labelled from A to L and β-strands are numbered from 1 to 10. The heme group, the bound androstenedione molecule at the active site and its polar interactions are shown.24, 25

Figure 3: Docking study of Standard aromatase Inhibitor Fadrazole with 3 EQM (Aromatase).
2. Aromatase inhibitors in the treatment of breast cancer

Estrogens can influence the risk of breast cancer and also the growth of established tumors. Hormone dependent breast tumors depends on the estrogens for growth. Two approaches for the treatment of breast cancer are either blocking the mechanism of action of estrogens or inhibition of its synthesis. These approaches are particularly helpful in postmenopausal women in whom the hormone response is common and estrogen synthesis is primarily peripheral (adipose tissue, muscle and breast tissue) rather than in the ovaries. A large number of aromatase inhibitors have been developed and utilized in clinical studies over the last 20 years. This development was prompted by the recognition of the fact that the cytochrome P450 inhibitor Aminoglutethimide is an aromatase inhibitor and exerts its therapeutic effectiveness in postmenopausal women with advanced breast cancer via the inhibition of aromatase.24, 25

Aromatase inhibitors are generally used clinically for metastatic hormonal dependent breast cancers, often in postmenopausal women when anti-estrogens become inefficient or are not well tolerated.26

2.1 Aromatase Inhibitors

Aromatase inhibitors which have been used clinically can be categorized on the basis of their generation and mechanism of action. They are described as first, second and third generation inhibitors according to the order of their clinical development. They can also be classified as type I and type II according to their mechanism of action. Type I and type II inhibitors are also known as steroidal and nonsteroidal inhibitors27, 28 respectively as shown in Table 1. The type I agents are either competitive inhibitors which are structurally related to the substrate or suicide inhibitors derived from androstenedione like 4-OHA or exemestane. The type II agents behave as competitive inhibitors, coordinating one of their heteroatoms (N, S, and O) to the iron in the heme of the cytochrome.

Table 1: Types of aromatase inhibitors

<table>
<thead>
<tr>
<th>Generation</th>
<th>Type-I Inhibitors (Steroidal)</th>
<th>Type-II Inhibitors (Nonsteroidal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>Testolectone</td>
<td>Aminoglutethimide</td>
</tr>
<tr>
<td>Second</td>
<td>Formestane</td>
<td>Fadrozole</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rogletimide</td>
</tr>
<tr>
<td>Third</td>
<td>Exemestane</td>
<td>Vorozole</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anastrozole</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Letrozole</td>
</tr>
</tbody>
</table>

2.1.1 Steroidal inhibitors (Type I)

The steroidal group includes inhibitors that acts in a competitive manner and certain others by enzyme inhibition or suicide inhibition.29, 30 A mechanism-based inhibitor is an inhibitor that mimics the substrate, which is converted by the enzyme to a reactive intermediate and results in the inactivation of the enzyme. The term “mechanism-based” is used because the inhibitors contains a chemical functionality that is acted upon by the enzyme during the normal catalytic process. A mechanism-based inhibitor produces time-dependent inactivation of the enzyme only
in the presence of catalytically active enzyme, omission of a cofactor, such as NADPH, does not produce inactivation. Other terms that are used for these inhibitors are “enzyme-activated irreversible inhibitors,” “suicide substrates,” and “suicide inhibitors” (Figure 4). Several mechanism-based aromatase inhibitors, structurally related to the natural substrate androstenedione, are initially recognized by the aromatase enzyme as alternate substrates and are then transformed (through an NADPH dependent mechanism) to reactive intermediates which bind irreversibly to the enzyme and produce inactivation.

![First Generation](Testolactone) ![Second Generation](Formestane) ![Third Generation](Exemestane)

**Figure 4:** Steroidal aromatase inhibitors

Mechanism-based inhibitors have distinct advantages in drug design, because these inhibitors are highly enzyme specific, produces prolonged inhibition and often exhibits minimum toxicities.\(^31, 32, 33\)

Competitive inhibitors are molecules that compete with the substrate androstenedione for noncovalent binding to the active site of the enzyme to decrease the amount of product formed. Such steroidal inhibitors have been developed upon the basic androstenedione nucleus by incorporating chemical substituents at varying positions of the steroid. These inhibitors bind to the aromatase cytochrome P450 enzyme in the same manner as the substrate androstenedione. Type I inhibitors associate with the substrate binding site of the enzyme and invariably have an androgen structure.\(^34\)

Steroidal inhibitors are aromatase substrate analogs having shape similarity to androstenedione, which is a more hydrophobic substrate than others, and presents the best affinity for the enzyme. Testolactone is used in breast cancer treatment for some 20 years and subsequently it has been reported to be a first generation aromatase inhibitor. Testololactone is a less potent member of the steroidal group of inhibitors and is infrequently used.\(^35\) Formestane (4-hydroxyandrostenedione; 4-OHA) is a structural analog of androstenedione and is thus a highly specific aromatase inhibitor.\(^36\) It was the first steroidal suicide-type (Type I) aromatase inhibitor to enter clinical trials. The 4-OHA molecule binds irreversibly to the enzyme active site and acts as a suicide inhibitor with prolonged action. That is why only a small dose of 4-OHA is needed for clinical treatment (250–500 mg intramuscularly every 2 weeks). Now it is a commercially available steroidal compound which is used as a second line of treatment for advanced breast cancer. Using the *in vitro* placental aromatase assay system, 4-OHA was shown to be 60-fold more potent than Aminoglutethimide (\(K_i=4.1 \mu M\)).\(^37\)
Exemestane is another steroidal inactivator (mechanism-based) which has been approved more recently for clinical use and it appears to be more potent than 4-OHA. Its $K_i$ for competitive inhibition is 10.2 nM and for irreversible inactivation 26 nM. Single dose administration causes a major reduction of plasma estrogens. A dose of 25 mg of Exemestane daily inhibited aromatase activity in patients expressing primary or secondary resistance to Aminoglutethimide. An advantage of exemestane is that it is orally active. The points of attachment of steroidal and non-steroidal aromatase inhibitors are shown in (Figure 5).

Figure 5: The points of attachment of steroidal and non-steroidal aromatase inhibitors.

2.1.2 Nonsteroidal inhibitors (Type 2)
Type II inhibition is usually occurs by reversible blockage of the estrogen, being dependent upon the continue presence of the drug. Non-steroidal inhibitors are mostly imidazole and triazole derivatives based on the structure of antifungal agents that inhibit P450 enzymes. Aminoglutethimide was the prototype of nonsteroidal inhibitor of aromatase. Aminoglutethimide was introduced as a form of medical adrenalectomy. The drug was given in sufficient doses to inhibit the production of adrenal steroids and replacement of corticoids are needed. Subsequently, however, it was shown that the Aminoglutethimide-corticoid regimen also can be block peripheral conversion of androgens to estrogen and suppressed circulating estrogens. Aminoglutethimide was the first inhibitor evaluated in clinical studies for the treatment of hormone-dependent breast cancer. Santen et al extensively evaluated the kinetic and hormonal effects of Aminoglutethimide in breast cancer and first proposed aromatase inhibition as the primary mechanism of action of Aminoglutethimide therapy. However, side effects like lethargy, ataxia and morbilliform rash and the development of more potent aromatase inhibitors resulted in cessation of further development of Aminoglutethimide for breast cancer.
Figure 6: First and second generation nonsteroidal aromatase inhibitors.

The imidazole compound Fadrozole is a potent, competitive inhibitor of aromatase, both in vitro and in vivo. Fadrozole, referred to as a “second-generation inhibitor,” is more selective than Aminogluthethimide and its inhibitory activity is 700 times more potent. However, this compound still has some nonselective inhibitory activity with respect to Aldosterone, Progesterone, and Corticosterone biosynthesis. (Figure 6) Clinical studies using Fadrozole proved that this nonsteroidal inhibitor is effective in the treatment of some postmenopausal women with advanced breast cancer. Structurally, majority of Type 2 inhibitors are Azoles (Figure 7). Many steroidal inhibitors lack specificity because a variety of steroid-metabolizing enzymes have cytochrome P450 prosthetic groups and these may also be inhibited. Reversible nonsteroidal anti-aromatase agents contains a suitable position of hetero atom that coordinates to heme iron P-450 enzyme. Because heme is a common moiety in all members of the CYP-450 super family, these inhibitors may lack specificity.

The third-generation inhibitors possess a specificity that appears to be nearly complete in clinical doses with no effect on Aldosterone, Progesterone, Corticosterone biosynthesis. Recent clinical studies have shown that aromatase inhibitors, especially the third-generation, are more effective than Tamoxifen in postmenopausal patients with metastatic breast cancer. A higher degree of specificity has been reached with the new generation of Triazole derivatives (Letrozole and Anastrozole). These newer agents are 100-3000 times more active than Aminogluthethimide and inhibit whole-body aromatization by a factor greater than 96%.

Figure 7: The third generation nonsteroidal aromatase inhibitors.

Letrozole (Femara, Novartis, Basel, Switzerland) is a potent nonsteroidal aromatase inhibitor that produces approximately 99% inhibition of estrogen biosynthesis at a dose of 2.5 mg/d in patients. Letrozole at a recommended therapeutic dose of 2.5 mg once daily effectively suppressed total-body aromatization, with a mean percentage inhibition of greater than 99.1%
and suppressed plasma estrone and estradiol levels by 84–88% in postmenopausal women with metastatic breast cancer.\textsuperscript{54}

Anastrozole (Arimidex, Astra-Zeneca, London, UK) is a potent nonsteroidal aromatase inhibitor which can be able to decrease the plasma estradiol levels in a dose-dependent manner and produce approximately 97\% inhibition of estrogen biosynthesis at a dose of 1 mg/d.\textsuperscript{55} Anastrozole at a recommended therapeutic dose of 1 mg once daily effectively suppressed total-body aromatization, with a mean percentage inhibition of 97.3\% and suppressed plasma estrone and estradiol levels by 81–85\% in postmenopausal women with metastatic breast cancer.\textsuperscript{56}

Vorozole is an another third generation non-steroidal oral aromatase inhibitor which is highly potent and specific for aromatase.\textsuperscript{57} Its clinical efficacy appears to be similar to that of Anastrozole and Letrozole. Vorozole has shown to be 1000-fold more potent than Aminogluthethimide in animal and human studies.\textsuperscript{58} However, Imidazole derivative Fadrozole is very rapidly metabolized \textit{in vivo}, the third generation clinical nonsteroidal aromatase inhibitors are all triazole compounds. \textit{In vitro} and \textit{in vivo} animal studies have shown its high potency and specificity.\textsuperscript{59} In a phase I clinical study of Vorozole racemates in postmenopausal women with advanced breast cancer, doses of 2.5-5 mg were well tolerated and resulted in suppression of estradiol to concentrations of <10 pmol/liter.\textsuperscript{60}

Overall, Anastrozole and Letrozole are more effective and have an advantage over standard therapy in terms of toxicity. Both of them are very potent and selective aromatase inhibitors and are well tolerated by patients.\textsuperscript{61, 62}

Apart from steroidal and nonsteroidal inhibitors, several flavones and isoflavones (Figure 8) have been demonstrated for the aromatase inhibitory activity. However, these natural products demonstrate numerous biological activities and interact with various enzymes and receptor systems of pharmacological significance, thus limiting their therapeutic usefulness. Considerable interests of flavonoids in breast cancer has arisen due to the hypothesis that these natural products present in soya and in rye flour, are dietary factors that may be responsible for the lower incidence of breast cancer in women from certain regions of the world.\textsuperscript{63, 64} Several flavonoids demonstrate aromatase inhibitory activities, thus lowering estrogen biosynthesis and circulating estrogen levels.\textsuperscript{65}

\textbf{Isoflavones}

\begin{center}
\begin{tabular}{c}
Biochanin A \\
\includegraphics[width=0.3\textwidth]{biochanin.png} \\
Synthetic pyridyl isoflavone analog \\
\includegraphics[width=0.3\textwidth]{synthetic.png}
\end{tabular}
\end{center}
Flavones

Chrysin

Apigenin

Flavone

Flavanone

Quercetin

7,8-Benzoflavone

Figure 8: Flavonoid derivatives as aromatase inhibitors.

2.2 Advantages and disadvantages of the two major classes of aromatase inhibitors.
A comparison of advantages and disadvantages of both of the categories of aromatase inhibitors are given in Table 2.66

Table 2: Comparison of Aromatase Inhibitors.

<table>
<thead>
<tr>
<th>Type of inhibitor</th>
<th>Advantages</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroidal Inhibitors</td>
<td>• More selective</td>
<td>• Poor oral bioavailability</td>
</tr>
<tr>
<td></td>
<td>• Longer half-life (inactivation feature)</td>
<td>• Androgenic effect on high dose</td>
</tr>
<tr>
<td></td>
<td>• Highly enzyme specific</td>
<td>• Metabolism by other CYP enzyme</td>
</tr>
<tr>
<td></td>
<td>• Prolonged inhibition</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Less toxic</td>
<td></td>
</tr>
<tr>
<td>Nonsteroidal inhibitors</td>
<td>• Less expected hormonal properties</td>
<td>• Less selective for enzyme</td>
</tr>
<tr>
<td></td>
<td>• Highly potent</td>
<td>• Musculoskeletal problems</td>
</tr>
<tr>
<td></td>
<td>• Good pharmacokinetic profile</td>
<td>• Shorter half life</td>
</tr>
<tr>
<td></td>
<td>• Oral bioavailability</td>
<td></td>
</tr>
</tbody>
</table>
2.3 Applications of Aromatase Inhibitors
Recently a number of clinical studies have been shown that all third generation aromatase inhibitors are superior to Tamoxifen therapy in term of efficacy and toxicity profile as a first line therapy for advanced breast cancer with metastasis. Aromatase inhibitors have been studied for treatment of breast cancer in its various stages, such as in advanced breast cancer, early stage of disease or its chemoprevention in highly susceptible women.67,68 Neo adjuvant therapy is to shrink the large primary tumors to make them operable, adjuvant therapy is to prevent recurrence of disease after surgical removal or radiation therapy of primary tumor. All third generation aromatase inhibitors are superior in neo adjuvant and adjuvant therapy than Tamoxifen. They also proved effectiveness in chemoprevention of disease in women which are at high risk of development of breast cancer. Recently the effectiveness and toxicity of aromatase inhibitors in pre-menopausal women with metastatic breast cancer has been reported.69

2.4 Steroidal Vs Nonsteroidal Aromatase Inhibitors
Although both steroidal and nonsteroidal aromatase inhibitors can block aromatase activity, there are distinct differences between the two classes. The differences related to the type of binding to the aromatase enzyme (irreversible or reversible binding for steroidal or nonsteroidal aromatase inhibitors, respectively) and potential androgenic effects of Exemestane, which may exert additional effects. Based on preclinical and clinical studies, combination therapy with the selective estrogen receptor modulator (SERM) Tamoxifen and the nonsteroidal aromatase inhibitor Anastrozole or Letrozole did not improve the antitumor efficacy of the nonsteroidal aromatase inhibitors alone.70 This result are in contrast with those of the steroidal aromatase inhibitors Exemestane and Tamoxifen, wherein the combination is more effective in reducing tumor growth than either of the agents alone in an experimental model.71 A mechanism explaining additive effects of the steroidal aromatase inhibitor Exemestane in combination with Tamoxifen has not been established.

The androgenic structure of type 1 steroidal aromatase inhibitors may give rise to hormonal effects apart from the decrease in estrogen production caused by inhibition of aromatase. The androgenic properties of Exemestane make it distinct from the nonsteroidal aromatase inhibitors Letrozole and Anastrozole.72

While considering the androgenic properties of aromatase inhibitors, it should be kept in mind that the differential effects of the type 1 drugs on healthy tissue may also affect clinical benefit for breast cancer patients. Work in model systems suggest that Exemestane treatment may lead to fewer adverse effects related to bone loss than nonsteroidal aromatase inhibitors because of its androgenicity.73, 74 Several clinical findings support a lower degree of bone loss with Exemestane.75 One might be expected that the steroidal structure of Exemestane may lend itself to an androgenic effect on bone that would be evident by a rise in bone formation markers (such as procollagen type 1 N-propeptide) not present with the nonsteroidal aromatase inhibitors.76 Cross resistance between steroidal and nonsteroidal aromatase inhibitors does not always occur. Breast cancer patients previously treated with the first-generation nonsteroidal aromatase inhibitor Aminogluthethimide subsequently respond to 4-hydroxyandrostenedione.77

2.5 Other Applications of Aromatase Inhibitors.
Aromatase inhibitors can be potentially used to treat a wide spectrum of excess estrogen related diseases. Several disorders in patients require estradiol to produce clinical manifestations and abrogation of the effects of this sex steroid ameliorates disease related signs and symptoms. Included in this list are hyperplasia and neoplasia of the breast and endometrium as well as gynecomastia, premature thelarche, precocious and delayed puberty, mastodynia, oligo- and anovulation, leiomyomata uteri and endometriosis. However, majority of the data on the pharmacological effectiveness of aromatase inhibitors come from their application for hormone dependent breast cancer in postmenopausal women. The observation of the 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. Studies with Aminoglutethimide generally revealed that estrogen synthesis is largely unaffected by the aromatase inhibitors, although increases in Gonadotrophins indicate a degree of compensated inhibition. Application of the more potent steroidal aromatase inhibitor, 4-OHA, even at a high dose of 500 mg/week, had no significant impact on premenopausal estrogen levels. In premenopausal women, the ability of reflex increments of LH and FSH to overcome the estrogen blockage of the ovary renders the aromatase inhibitors inactive when used alone. Due to an insufficient ovarian blockage in premenopausal women with breast cancer, the aromatase inhibitors must be combined with a GnRH agonist to prevent the reflex LH and FSH increments. After menopause, estrogens are synthesized in extragonadal tissues from adrenal androgens mainly by adipose and muscle tissues, because these tissues make up a large proportion of the body mass. Estrogen concentrations in tumors are higher than in breast fat suggesting that estrogens produced within the breast may be critical in stimulating tumor proliferation. Thus, effective inhibition of breast aromatase could be an important determinant of the outcome of aromatase inhibitor treatment for breast cancer. In postmenopausal women, the aromatase inhibitors can be block estradiol production directly in the breast as well as in fats tissue. LH and FSH do not regulate extraglandular aromatase or substrate levels and thus suppression of estradiol is nearly complete. The application of aromatase inhibitors for hormone dependent breast cancer in postmenopausal women has been described in several review papers.

Table 3: Applications and side effects of currently available aromatase inhibitors

<table>
<thead>
<tr>
<th>Generation</th>
<th>Clinical Applications</th>
<th>Common Side Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Generation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aminoglutethimide</td>
<td>Medical adrenalectomy in breast cancer.</td>
<td>Drug rash, fever and lethargy.</td>
</tr>
<tr>
<td>Testolactone</td>
<td>It is used to treat advanced stage breast cancer in women who have been through menopause or whose ovaries no longer function.</td>
<td>Abnormal skin sensations, aches of the legs and arms, general body discomfort, hair loss, loss of appetite, nausea, redness of the tongue and vomiting.</td>
</tr>
<tr>
<td>Generation</td>
<td>Drug</td>
<td>Indication</td>
</tr>
<tr>
<td>------------------</td>
<td>---------------</td>
<td>----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Second Generation</td>
<td>Fadrozole</td>
<td>In the treatment of patients with post-menopausal breast cancer.</td>
</tr>
<tr>
<td></td>
<td>Formestane</td>
<td>In postmenopausal women with breast cancer.</td>
</tr>
<tr>
<td>Third Generation</td>
<td>Anastrozole</td>
<td>Post-menopausal women diagnosed with hormone-receptor-positive, early-stage breast cancer after surgery.</td>
</tr>
<tr>
<td></td>
<td>Letrozole</td>
<td>To treat hormonally-responsive breast cancer &amp; is effective only in post-menopausal women.</td>
</tr>
<tr>
<td></td>
<td>Vorozole</td>
<td>To treat advanced postmenopausal breast cancer following Tamoxifen failure.</td>
</tr>
<tr>
<td></td>
<td>Exemestane</td>
<td>In the treatment of advanced breast cancer in postmenopausal women whose disease has progressed following Tamoxifen therapy.</td>
</tr>
</tbody>
</table>

2.6 Side Effects of Aromatase Inhibitors

Aromatase inhibitors can completely block the estrogens synthesis, as a result they also inhibit beneficial effects of estrogens on bones and lipid profile. They can increases the chances of osteoporosis, bone fracture (specifically fractures of spine, hip and wrist) and increase cholesterol, triglycerides, low density lipoprotein levels, and decrease protective high density lipoproteins level. In this way they increase chances of cardiovascular diseases. However, in this regard SERMs are superior in that they exert beneficial effects on bones and lipid profile and antiestrogenic effect in the breast.

Aromatase inhibitors tend to cause fewer serious side effects than Tamoxifen, such as blood clots, stroke and endometrial cancer. The most common side effects of aromatase inhibitors are...
Joint stiffness or joint pain. Joint pain from taking an aromatase inhibitor can be troubling. But British study which has been conducted in 2008 suggest that women who experience joint pain while taking hormonal therapy will be less likely to have the breast cancer come back.\textsuperscript{89} Hot flushes have been reported with the use of selective aromatase inhibitors but their incidences seem to be comparable with that of Tamoxifen. In hematological system Aminogluthethimide causes leucopenia owing to its activation to cytotoxic metabolites by hepatic CYP 450 enzymes.\textsuperscript{90} Brief clinical applications and common side effects of aromatase inhibitors are given in\textsuperscript{91,92} Table 3

2.7 Future Developments

There is a need for emergence of fourth generation aromatase inhibitors with specific aromatase inhibition and low toxicity profiles especially for addressing musculoskeletal disorders. There should be more clinical trials of existing aromatase inhibitors to resolve problems like role of aromatase inhibitors in chemoprevention and clinical response among the third generation agents. In future clinical studies it is important to test ER receptor status, receptor coactivators and ErbB-2 for better treatment approach. Combination therapy of aromatase inhibitors along with agents targeting other molecular targets like COX-2 inhibitors may have impact on efficacy of aromatase inhibitors, recently some studies have been planned for this purpose.

3. CONCLUSION

With the progress in molecular endocrinology of estrogens and medicinal chemistry of aromatase inhibitors, we have different strategies for inhibition of aromatase and different classes of chemical compounds to treat estrogen dependent diseases. Availability of large number and different classes of aromatase inhibitors aided to design optimal pharmacophore pattern to inhibit aromatase. The third generation aromatase inhibitors are both remarkably potent and specific endocrine agents inhibiting aromatase activity. Aromatase inhibitors have also been used in the neoadjuvant setting, where they have been shown to achieve higher response rates than Tamoxifen and decrease the need of major surgery. Finally, though third generation aromatase inhibitors are emerging as potential endocrine agents for the treatment of breast cancer, there is plenty of scope and need for improvement.

4. ACKNOWLEDGMENT

The authors are thankful to the Head, Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur for providing necessary facilities and other technical supports during the preparation of this review article. The authors Priyanka and Shweta are also heartily thankful to the All India Council for Technical Education, New Delhi for the financial assistance in the form of fellowship.

5. CONFLICT OF INTEREST STATEMENT

The authors report no conflict of interest.
6. REFERENCES


