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Aromatase research and its clinical significance

Znaczenie kliniczne badań nad aromatazą

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Abstract

Aromatase is a member of the cytochrome P450 superfamily that catalyzes the conversion of androgens (C_{19}), namely testosterone and androstenedione, into oestrogens (C_{18}), oestradiol, and oestrone, respectively. The enzyme is active in various tissues in both females and males, thus oestrogens are produced not only in gonads but also in extra-gonadal localizations such as brain, adipose tissue, breast, skin, and bone. Aromatase gene *CYP19A1* located on chromosome 15 comprises nine coding exons and a number of alternative non-coding first exons that regulate tissue-specific expression. Studies on local regulation of aromatase expression and activity are important for understanding processes such as growth of oestrogen-dependent breast cancer. Rare clinical conditions of aromatase deficiency and excess have revealed some new and unexpected oestrogen functions in metabolism and bone health in both women and men. They were further studied using transgenic animal models such as aromatase knockout mice (ArKO) or (AROM+) mice overexpressing human aromatase. Research on aromatase was important for its practical outcome as it contributed to the development of aromatase inhibitors (AIs), an effective and safe group of drugs for the first-line endocrine therapy of breast cancer. Further studies are needed to establish AIs application in other oestrogen-dependent conditions, to overcome the resistance in breast cancer patients, and to develop tissue-specific selective inhibitors. **(Pol J Endocrinol 2010; 61 (1): 126–134)**

Key words: *aromatase, oestrogens, androgens, aromatase inhibitors, breast cancer*

Streszczenie

Aromataza jest enzymem należącym do rodziny cytochromu P450. Katalizuje reakcję hydroksylacji prowadzącą do powstania estrogenów: estradiolu i estronu z androgenowych substratów, odpowiednio: testosteronu i androstendionu. Aktywność enzymu i produkcję estrogenów wykazano w różnych tkankach zarówno u kobiet, jak i u mężczyzn. Poza gonadami aromataza jest aktywna na przykład w mózgu, tkance tłuszczowej, gruczole piersiowym, skórze i kościach. Gen aromatazy *CYP19A1*, zlokalizowany na chromosomie 15, składa się z dziewięciu kodujących egzonów i alternatywnych niekodujących pierwszych egzonów, których swoista tkankowo transkrypcja reguluje ekspresję genu. Poznanie mechanizmów regulujących lokalną ekspresję i aktywność aromatazy przyczynia się między innymi do lepszego zrozumienia procesów istotnych dla rozwoju estrogenozależnego raka piersi. Opisy klinicznych przypadków niedoboru i nadmiaru aromatazy oraz analiza fenotypu myszy transgenicznych pozbawionych aromatazy (ArKO) lub z jej nadekspresją (AROM+) ujawniły dotychczas nieznane i często zaskakujące funkcje estrogenów u obu płci. Badania podstawowe nad aromatazą znalazły swoje praktyczne zastosowanie w pracach nad inhibitorami aromatazy. Stanowią one obecnie pierwszoplanowe leczenie hormonalne raka piersi kobiet po menopauzie, w przypadku obecności receptorów estrogenowych w komórkach guza. Potrzebne są dalsze badania nad zastosowaniem inhibitorów aromatazy w innych schorzeniach zależnych od estrogenów, nad przeciwdziałaniem rozwojowi oporności powstającej w trakcie terapii oraz opracowaniem selektywnych inhibitorów swoistych tkankowo. **(Endokrynol Pol 2010; 61 (1): 126–134)**

Słowa kluczowe*: aromataza, estrogeny, androgeny, inhibitory aromatazy, rak piersi*

Aromatase — the key enzyme in oestrogen biosynthesis

Aromatase is a member of the cytochrome P450 superfamily, a very large family of enzymes catalyzing incorporation of an atom of oxygen into an organic molecule, called hydroxylases. These widespread membrane-bound proteins are present in most animal, plant, and human tissues, such as the liver, and they are essential for the synthesis of cholesterol and steroid hormones and metabolism of xenobiotics and fatty acids. The common feature of the family is the presence of heme as a prosthetic group that forms the active site. It contains iron that can undergo oxidation and reduction. Reduced nicotamide adenine dinucleotide phosphate (NADPH) is required as a coenzyme for the reactions catalyzed by cytochrome P450 enzymes. The name of the group is taken from the wavelength of maximum light absorption at 450 nm of the ferrous carbon monoxide complex [1–2].

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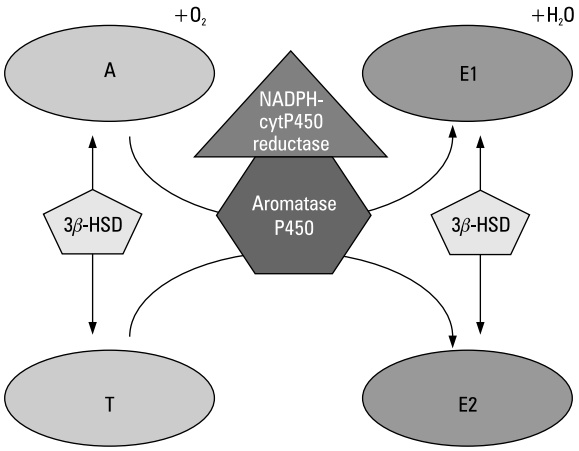

Figure 1. *Schematic diagram of the reaction catalyzed by aromatase enzyme complex. A — androstenedione, E1 — oestrone, T — testosterone, E2 — oestradiol, 3β-HSD — 3β-hydroxysteroid dehydrogenase NADPH — reduced nicotinamide adenine dinucleotide phosphate, cytP450 — cytochrome P450*

Rycina 1. *Schemata reakcji katalizowanych przez kompleks enzymatyczny aromatazy. A — androstendion, E1 — estron, T — testosteron, E2 — estradiol, 3β-HSD — dehydrogenaza 3β-hydroksysteroidowa, NADPH — zredukowany dinukleotyd nikotynoamidoadeninowy, cytP450 — cytochrom P450*

Human aromatase is a 58 kDa protein that was purified from placental microsomes in the 1980s [3–6]. The protein is highly conserved among all vertebrates. In the enzymatic complex with flavoprotein, NADPH-cytochrome P450 reductase, it catalyzes a complex reaction sequence that results in the conversion of androgens (C_{19}), namely testosterone and androstenedione, into oestrogens (C_{18}), oestradiol, and oestrone, respectively [7–9] (Fig. 1).

Many different cell types have been shown to express aromatase: granulosa cells, Leydig and Sertoli cells, placental cells, neurons, preadipocytes and fibroblasts, vasculature smooth muscle cells, chondrocytes, and osteoblasts [4–6, 10–18]. Thus, oestrogens are produced in various tissues, not only in those traditionally known to be involved in that process, such as gonads — ovaries and testes or placenta, but also brain, adipose tissue, breast, skin, blood vessels, bone, and cartilage [19]. Expression levels show interpersonal and regional differences, and they are different at various stages of life, e.g. foetal liver expresses aromatase, but it is not present in adult liver [9]. In women of reproductive age, the ovaries express high levels of aromatase and they are the main source of oestrogens. After menopause peripheral tissues become sites of oestrogen synthesis of great importance. Similarly, in men,85% of oestradiol and more than 95% of oestrone is produced in extraglandular tissues as a result of aromatisation of circulating androgens [19–20]. Oestrogens synthesized locally in extragonadal sites mostly do not enter the circulation but exert intracrine, autocrine, paracrine, and juxtacrine effects, acting directly in the cells of synthesis or on the neighbouring cells [16]. These interactions at the tissue level are very difficult to measure in the clinical setting, and they remain mostly unrecognized. However, it is believed nowadays that in the post-menopausal breast, it is local oestrogen synthesis that determines their tissue level and affects breast cancer risk [21].

Aromatase is encoded by a single copy of the *CYP19A1* gene located on the short arm of chromosome 15 (15q21) [22–23]. It is approximately 120 kb long and comprises 10 exons. Nine coding exons (II-X) span approximately 30kb, and there are a number of alternative non-coding first exons which are expressed in a tissue-specific manner. As various tissues utilize their own promoters and associated enhancers and suppressors, the tissue-specific regulation of oestrogen synthesis is very complex. Although the transcripts for aromatase have different 5′ ends in various tissues depending on the promoter usage, these unique first exons are spliced into a common 3′-junction upstream of the start of translation, resulting in the synthesis of identical aromatase proteins [11, 24–25]. Thus, use of alternative promoters does not affect protein structure but its expression level.

The first described distal promoter I.1, located approximately 89 kb upstream of exon II, drives transcription in the placenta. On the other hand, the proximal promoter found immediately upstream of exon II is the main one utilized in the gonads. In between these two, several other first exons and promoters have been identified, such as I.2 — placental minor, I.3 in adipose tissue and breast cancer, I.4 in skin fibroblasts, preadipocytes, and bone, I.5 — foetal, I.6 in bone, I.7 — epithelial and overexpressed in breast cancer, and I.f in the brain [10–11, 24–28].

Adipose promoter I.4 drives aromatase transcription in normal adipose mesenchymal cells at a relatively low level. Class 1 cytokines (IL-6, oncostatin M, and IL-11) and TNF-α produced locally within the adipocytes are major factors regulating this promoter, while promoter II is regulated by cAMP and gonadotropins. However, in the presence of breast cancer secreting numerous regulatory factors, the adipose stromal cells can start predominantly utilizing promoter II, together with promoters I.3 and I.7. The switch of the promoters depending on the tissue microenvironment results in enhancement of aromatase gene transcription, protein expression, and its enzymatic activity compared to the normal breast tissue. This process is the primary reason for the increased oestrogen production in adipose stromal cells surrounding the breast cancer [28–30]. Many breast tumours can overexpress cyclooxygenase-2 (COX-2)

producing and secreting prostaglandin E2 (PGE2), a powerful stimulator of aromatase expression acting through cAMP that regulates transcription from promoter II in adjacent preadipocytes. Moreover, COX-2 inhibitors have been shown to inhibit aromatase activity of breast cancer cells [31]. A similar process may be involved in the development of idiopathic gynaecomastia. It was shown that in the active phase of breast tissue proliferation (florid-type gynaecomastia) mRNA expression of aromatase from promoter II was significantly increased compared to that in the fibrous type, such as expression of COX-2 [32].

Aromatase deficiency

Aromatase deficiency is a very rare autosomal recessive disorder which has been described in less than 20 cases to date [33–46]. It results from various mutations in the coding region of the *CYP19A1* gene that lead to a decrease or loss of enzyme function, and as a result oestrogen deficiency. Most of the reported mutations are single-base changes in exons IX and X, important for substrate binding and encoding the heme-binding region. Such mutations result in codon changes and single amino acid substitutions or premature stop-codons leading to the production of a truncated protein. One of them destroyed an exon-intron splice junction and resulted in an in-frame insertion of amino acids within the coding region [34]. The majority of subjects were homozygous for inactivating mutations because of pregnancies from consanguineous parents, but some were compound heterozygotes from non-related parents. Describing patients with aromatase deficiency and generation of aromatase knock-out mice (ArKO) enabled detailed study of some new and unexpected oestrogen functions in both female and male organisms, not only those related to reproduction [47].

An animal model of aromatase deficiency was produced by disruption of exon IX, which led to enzyme inactivation and inability to synthesize oestrogens [48]. Visibly, the phenotype of ArKO mice is characterized by age-progressing obesity, with the excessive accumulation of intra-abdominal fat. Obesity is associated with increased circulating lipids and hyperinsulinaemia with insulin resistance. Although the metabolic changes were present in both sexes, only male ArKO mice were found to develop liver steatosis that could reverse after beta-oestradiol treatment [49]. In both sexes, loss of the bone mineral density was observed. There are distinctive reproductive phenotypes with female infertility caused by anovulation, dysmorphic, and degenerative ovaries with numerous haemorrhagic cysts in the follicles and severely undeveloped uteri. In male ArKO mice, fertility is also compromised by disruption in spermatogenesis and impaired sexual behaviour [48–54].

In humans, the first case of aromatase deficiency was described in 1995 [33], which was much later than deficiencies in other enzymes of steroidogenic pathways. In both sexes, the first symptoms appear before birth in the pregnant mothers that develop progressive virilisation due to an inability to aromatize androgens by the placenta. Excessive androgen levels in utero results in androgenisation of the female foetuses, seen as the presence of ambiguous genitalia at birth. Thus in all cases of female pseudohermaphroditism with 46XX genotype and normal female internal genitalia and ovaries present, a diagnosis of aromatase deficiency should be considered. The childhood could be unaffected or there might be symptoms of haemorrhagic cysts of the ovaries. At the time of puberty a normal adrenarche is present; however, there is primary amenorrhea and lack of breast development. Hormonal tests show hypergonadotropic hypogonadism with hyperandrogenism. As a result of androgen excess, acne and hirsutism could appear and the virilisation might progress with age. The bone age is delayed because oestrogens are crucial for epiphyseal closure, and decreased bone mineral density could be observed [33, 35–37, 39, 41, 44]. Only recently, the first case of an adult aromatase deficient female not treated with oestrogen replacement therapy since puberty was reported. The results of the study showed severe bone and joint changes and metabolic phenotype similar to that observed in men with exception of liver steatosis [55]. Some authors notice that the phenotypes could be variable or "non-classic", for example with some degree of breast and uterine development, depending on some residual aromatase activity and probable differences in oestrogen and androgen responsiveness [44].

In males, unlike in females, symptoms of aromatase deficiency appear after puberty. The most characteristic is progressive linear growth into adulthood caused by the inability of the growth plates to fuse without oestrogen action, regardless of the relatively high levels of testosterone. The findings included also *genu valgum* or knocked knees, eunuchoid body proportions, delayed bone age, osteopenia, and osteoporosis. The observed bone phenotype confirms a major role for oestrogens in the maintenance of bone mass and bone maturation in men. Similarly to male ArKO mice, male patients with aromatase deficiency are obese. Metabolic syndrome characterized by abdominal obesity, dyslipidemia, hyperinsulinaemia, and acanthosis nigricans, as a symptom of insulin resistance, glucose intolerance, or diabetes mellitus might develop and progress with age. Fatty livers were described in some patients. Impaired

fertility and low libido might be present and cryptorchidism was reported [37, 38, 40, 42–47]. Hormonal analyses revealed undetectable oestradiol and oestrone levels and elevated gonadotropins, while androstenedione and testosterone levels could be elevated or within normal range, indicative that oestrogens regulate FSH and LH secretion in the negative feedback loop in men [56].

The phenotype of aromatase deficient males is similar to that observed in the man reported with an inactivating mutation in the oestrogen receptor α [57]. Unlike in oestrogen resistance, men with aromatase deficiency treated with exogenous oestrogens showed a significant improvement of the clinical picture [38, 42, 46, 58]. Positive effects of oestrogen therapy, with no effect of androgen therapy, on bone maturation, mineral density, and blood lipid concentrations were described, and improvement of insulin resistance, hyperglycaemia, and liver steatohepatitis was shown in some cases.

Aromatase excess

Transgenic mice expressing human aromatase under the human ubiquitin C promoter (AROM+) or MMTV-arom+ mice overexpressing aromatase locally in breast tissue are both very valuable tools to analyze processes regulated by oestrogens. They help in understanding molecular mechanisms involved in the formation of the mammary glands, gynaecomastia, and breast cancer [59-60]. Local expression of aromatase in MMTV-arom+ mice does not significantly influence the circulating oestrogen levels, while in AROM(+) mice, serum estradiol is elevated together with prolactin, and the testosterone level is reduced. In males of both strains, development of mammary glands (gynaecomastia) is observed, and the process could be stopped or reversed with aromatase inhibitor treatment [61].

There are few families described in the literature with the oestrogen excess syndrome due to aromatase overexpression [62–68]. It is characterized by severe prepubertal gynaecomastia in males and macromastia, premature puberty, enlarged uterus, and menstrual irregularities in females. Premature fusion of growth plates and short final stature is present in both sexes. Increased aromatization of androgens leads to high serum oestradiol and oestrone levels with low testosterone and androstenedione and suppressed secretion of gonadotropins. Aromatase inhibitors were effective in the treatment of these patients [65].

Binder et al. studied a family with seven men in three generations affected with gynaecomastia that was inherited in an autosomal dominant pattern [67]. Their testosterone levels were decreased while oestrone and oestradiol were in the high normal range. A strong association of the TTTA repeat polymorphism in the *CYP19A1* gene was observed. In a similar family, the same molecular marker associated with the phenotype was reported by Stratakis et al. [64]. Increased aromatase activity in fibroblasts and strong immunostaining for aromatase in breast tissue samples from family members with gynaecomastia were shown. A new promoter was revealed in the non-coding region of the *CYP19A1* gene. Its activation could possibly lead to the increased aromatase gene expression.

Other alterations in the promoter region of *CYP19A1* were described by Shozu et al. [65]. In three men with severe gynaecomastia of prepubertal onset and hypogonadotrophic hypogonadism resulting from severe oestrogen excess, two novel gain-of function mutations led to the overexpression of aromatase in many tissues. Heterozygous inversions in the 15q21.2–3 region caused the constitutively active cryptic promoters that normally serve to transcribe two ubiquitously expressed genes — *FLJ* or *TMOD3* — to lie adjacent to the aromatase coding region. Similar regional rearrangements resulting in the formation of cryptic promoters for aromatase gene and its overexpression were described recently by Demura et al. [68].

Different rearrangements in the upstream region of the aromatase gene and the use of alternative more active promoters might happen relatively often and sometimes cause subtle symptoms of oestrogen excess, and thus be unrecognized. Locally enhanced oestrogen production is impossible to be measured in normal clinical conditions, as it may not significantly affect the circulating hormones. However, it is thought that even subtle local aromatase overexpression may increase the risk of oestrogen dependent conditions, such as breast and endometrial cancer, endometriosis, gynaecomastia, and macromastia [69].

Molecular mechanisms involved in some physiologic or pathologic processes linked to enhanced conversion of androgens to oestrogens still need to be studied. Increased aromatase activity was shown in aging [70–71], obesity [70], hyperthyroidism [72], idiopathic gynaecomastia [32, 73], and tumours [74–80] such as testicular tumours, adrenocortical carcinoma, fibrolamellar hepatocellular carcinoma, giant cell carcinoma of the lung, and melanoma.

Aromatase gene polymorphisms

Apart from the rare mutations affecting the *CYP19A1* gene, more frequent genetic changes called polymorphisms can appear. These are changes in DNA sequence that occur in more than 1% of the population causing the occurrence of different allelic forms of a gene. They are inherited and their configuration is unique for every person.

Table I. *Aromatase inhibitors*

Tabela I. *Inhibitory aromatazy*

First generation	Aminoglutethimide*	Tabl a 250 mg	Cytadren, Aminoglutetymid, Orimeten
	Testolactone*	Tabl a 50 mg	Fudestrin, Teslac
Second generation	Formestane* (4-hydroxyandrostenedione)	Amp a 250 mg	Lentardon depot
	Fadrozole*	Tabl a 1 mg	Afema
Third generation	**Exemestane**	Tabl a 25 mg	Aromasin
	Anastrozole	Tabl a 1 mg	Anastralan, AnastroLek, Anastrozol-ratiopharm, Ansyn, Arimidex, Atrozol, Egistrozol, Symanastrol
	Letrozole	Tabl a 2.5 mg	Aromek, Femara, Lametta, Letrozol Teva
	Vorozole*	Tabl a 2.5 mg	Rivizor

*not registered for sale in Poland

More than 80 genetic polymorphisms in the *CYP19A1* gene have been described [81]. Most of them are single nucleotide polymorphisms (SNPs) that are present in non-coding regions or they are coding but synonymous. There are only four non-synonymous coding SNPs: $Trp^{39}Arg$ and $Thr^{201}Met$, which do not affect aromatase activity, and $Arg^{264}Cys$ and $Met^{364}Thr$, which decrease its enzymatic function. Probably the most extensively studied are intron IV polymorphisms: a microsatellite $(TTTA)_n$ repeat at position 77, and the TCT insertion/deletion at position 27. Published data suggest the association of these polymorphisms with aromatase activity, sex hormones levels [82–85], and oestrogen-dependent conditions such as breast cancer [84, 86–88], osteoporosis [82–83, 89], endometrial cancer [90–91], endometriosis [92–93], prostatic cancer [94–95], and gynaecomastia [64, 67, 96]. Generally, long alleles of the $(TTTA)_n$ polymorphism with more than 8 or 10 repeats and the TCT insertion were associated with increased incidence of hyperoestrogenic conditions. However, some results are conflicting. It is also not clear what the molecular mechanisms are, as the polymorphisms are found in the intronic sequence. They could be markers that are in linkage with some other functional polymorphisms, or they might influence the level of gene expression. The *in vitro* studies of Gennari et al. confirmed increased aromatase activity in skin fibroblasts from subjects with a high-repeat genotype with more than nine TTTA repeats [82]. Another study by Berstein et al. showed the highest aromatase activity in endometrial tumours from patients with $(TTTA)_{11}/(TTTA)_{11}$ and $(TTTA)_{11}/(TTTA)_{12}$ genotypes [90].

Aromatase inhibitors

More than a century ago, in 1896, bilateral oophorectomy was first shown to be an effective treatment for advanced breast cancer in pre-menopausal women [97]. Since then, different methods of treatment with the intent of oestrogen deprivation were introduced, such as hypophysectomy, surgical and then pharmacological adrenalectomy, selective oestrogen receptor blockage with tamoxifen, and finally the use of potent aromatase inhibitors [98–100].

Aromatase inhibitors (AIs) form a group of drugs that have the ability to cease the production of oestrogens by inhibiting their conversion from androgens (Table I). AIs are used in the endocrine therapy of breast cancer expressing oestrogen receptors (ER) in postmenopausal women because local oestrogens produced in the tumour and surrounding cells are major stimulants for the cancer growth in these patients [21, 28–30, 100–102].

Aminoglutethimide (AG), used firstly as an inhibitor of adrenal steroidogenesis for pharmacological adrenalectomy, was later found to block total body aromatisation and was rediscovered as a non-selective first generation aromatase inhibitor [103]. However, its use in breast cancer patients was limited due to numerous side effects and the necessity to substitute adrenal steroids with dexamethasone or hydrocortisone. Another first-generation aromatase inhibitor, used for advanced breast cancer treatment even before that mechanism of action was demonstrated, was testolactone [104]. Later in the 1970s, tamoxifen was introduced, and after proving to be effective and safer than AG, it became the first line endocrine therapy for breast cancer [98–99, 105]. At the same time, work on developing selective aromatase inhibitors continued.

The first selective steroidal, suicide inhibitor in the second generation of AIs was 4-hydroxyandrostenedione or formestane. It became available for postmenopausal breast cancer treatment in the 1980s in intramuscular injections. Used as a second line therapy after tamoxifen, it was soon proven comparably effective, and the

side effects were improved compared to the first generation AIs [106–107]. Another second generation AI was fadrozole [108]. It was classified as a type 2 inhibitor, being a non-steroidal compound binding reversibly to the heme group of the enzyme, as opposed to irreversibly binding steroidal analogues of androstenedione (type 1 inhibitors), called enzyme inactivators. Fadrozole use was restricted by its rapid clearance and inhibition of aldosterone synthesis observed with the doses needed for effective aromatase inhibition [109].

The 1990s brought the discovery of the highly potent selective third generation aromatase inhibitors: exemestane (steroidal type 1 inhibitor), anastrozole, and letrozole (triazoles, type 2 inhibitors) [100]. They were shown to have higher potency, specificity, greater efficacy, and less toxicity than first and second generation drugs [110–112]. Whole body aromatization measurements revealed that the mean degree of enzyme inhibition is more than 97% [113]. The results from the large multi-centre randomized trials comparing third generation AIs to tamoxifen demonstrated their superiority over tamoxifen in the treatment of advanced postmenopausal breast cancer with respect to efficacy and safety [114–11]). Subsequently, trials of anastrozole (ATAC) or letrozole (BIG 1-98 FEMTA) versus tamoxifen as an adjuvant therapy for early stage cancer, given after initial surgery to prevent recurrence, showed positive results for AIs and led to the approval of third generation aromatase inhibitors for such use [117–119]. In the neo-adjuvant setting, used before surgical treatment, AIs reduced tumour size more effectively than tamoxifen [116, 120].

Aromatase inhibitor side effects differ from those observed with SERM treatment. While tamoxifen increases endometrial cancer incidence and risk of deep venous thromboses and pulmonary emboli, AIs are mainly associated with accelerated bone loss leading to osteopenia and osteoporosis, increased risk of fractures, arthralgias, and symptoms of urogenital atrophy. The frequency of hot flushes, headache, and gastrointestinal symptoms is comparable [114–121].

Currently registered indications for anastrozole include advanced postmenopausal breast cancer treatment (even ER-negative if there is an initial response to tamoxifen) and adjuvant therapy of ER-positive cancers. Letrozole could be used as a first line therapy in both advanced and adjuvant settings with ER-positive tumours, or could be introduced after an initial 5 years of treatment with tamoxifen. They are preferred to SERMs, especially in patients with high risk of veno-thrombotic episodes and those with localised hormone-receptor-positive breast tumours [118]. Exemestane is registered for post-tamoxifen treatment of advanced disease.

The side effects observed during treatment with the phase III inhibitors are the result of global inhibition of the catalytic activity of aromatase in all tissues; thus, the perfect approach would be the blockage of oestrogen production only in the breast. The development of tissue-selective inhibitors of aromatase expression is plausible because of tissue-specific regulation of *CYP19A1* expression, and it is an extensively researched subject. A blockade of promoter II/I.3-mediated transcription might provide a breast-specific therapy, thus factors regulating transcription from those promoters are being studied. [122]. Other studies concentrate on the mechanisms of resistance to aromatase inhibitors and the ways of overcoming that process [123–124].

Apart from their primary application in breast cancer, aromatase inhibitors are being experimentally used in gynaecology and paediatrics, *e.g.* induction of ovulation in anovulatory patients, endometriosis, short stature, and gynaecomastia [20, 65, 67, 125–129]. Some of these data are promising, but they need to be confirmed in larger randomized, controlled trials.

Conclusions

Years of research in the field of aromatase — on its function, its gene and structure, complex regulation, and means of pharmacological inhibition — have led to some great discoveries and breakthroughs. Describing the clinical conditions caused by aromatase excess or deficiency and producing animal models has demonstrated new and unexpected roles of oestrogens for both women and men, in many physiological and pathological processes, not only those related to reproduction. The dogma that oestrogens are solely female sex hormones was refuted. Most importantly, the basic research contributed to the origin of an important group of drugs: the aromatase inhibitors that are now the most effective and safe endocrine treatment for breast cancer. Further research could lead to the development of more potent and specific agents and to finding and proving the efficacy for some new applications.

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