Review

The selective estrogen enzyme modulators in breast cancer: a review

Jorge R. Pasqualini*

Hormones and Cancer Research Unit, Institut de Puériculture, 26 Boulevard Brune, 75014 Paris, France

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Abstract

It is well established that increased exposure to estradiol (E2) is an important risk factor for the genesis and evolution of breast tumors, most of which (approximately 95–97%) in their early stage are estrogen-sensitive. However, two thirds of breast cancers occur during the postmenopausal period when the ovaries have ceased to be functional. Despite the low levels of circulating estrogens, the tissular concentrations of these hormones are significantly higher than those found in the plasma or in the area of the breast considered as normal tissue, suggesting a specific tumoral biosynthesis and accumulation of these hormones. Several factors could be implicated in this process, including higher uptake of steroids from plasma and local formation of the potent E2 by the breast cancer tissue itself. This information extends the concept of ‘intracrinology’ where a hormone can have its biological response in the same organ where it is produced. There is substantial information that mammary cancer tissue contains all the enzymes responsible for the local biosynthesis of E2 from circulating precursors. Two principal pathways are implicated in the last steps of E2 formation in breast cancer tissues: the ‘aromatase pathway’ which transforms androgens into estrogens, and the ‘sulfatase pathway’ which converts estrone sulfate (E1S) into E1 by the estrone-sulfatase. The final step of steroidogenesis is the conversion of the weak E1 to the potent biologically active E2 by the action of a reductive 17β-hydroxysteroid dehydrogenase type 1 activity (17β-HSD-1). Quantitative evaluation indicates that in human breast tumor E1S ‘via sulfatase’ is a much more likely precursor for E2 than is androgens ‘via aromatase’. Human breast cancer tissue contains all the enzymes (estrone sulfatase, 17β-hydroxysteroid dehydrogenase, aromatase) involved in the last steps of E2 biosynthesis. This tissue also contains sulfotransferase for the formation of the biologically inactive estrogen sulfates. In recent years, it was demonstrated that various progestins (promegestone, nomegestrol acetate, medrogestone, dydrogesterone, norelgestromin), tibolone and its metabolites, as well as other steroidal (e.g. sulfamates) and non-steroidal compounds, are potent sulfatase inhibitors. Various progestins can also block 17β-hydroxysteroid dehydrogenase activities. In other studies, it was shown that medrogestone, nomegestrel acetate, promegestone or tibolone can stimulate the sulfotransferase activity for the local production of estrogen sulfates. All these data, in addition to numerous agents which can block the aromatase action, lead to the new concept of ‘Selective Estrogen Enzyme Modulators’ (SEEM) which can largely apply to breast cancer tissue. The exploration of various progestins and other active agents in trials with breast cancer patients, showing an inhibitory effect on sulfatase and 17β-hydroxysteroid dehydrogenase, or a stimulatory effect on sulfotransferase and consequently on the levels of tissular levels of E2, will provide a new possibility in the treatment of this disease.

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1. Introduction

In Western countries (Europe, USA, Canada, South America) breast cancer represents 25–30% of the total incidence of cancers in women and accounts for 15–18% of mortality.

The risk of a woman developing breast cancer during her lifetime is 1 in 8 in the United States, 1 in 12 in the European Community and 1 in 80 in Japan. Two-thirds of breast cancers are detected in postmenopausal women.

Most breast cancers (about 95%), whether in pre- or postmenopausal women, are initially hormone-dependent, where the hormone estradiol plays a crucial role in their development and progression [1–4]. The hormone and estrogen receptor (ER) complex can mediate the activation of proto-oncogenes and oncogenes (e.g. c-fos, c-myc),
nuclear proteins, as well as other target genes. Consequently, processes that modulate the intracellular concentrations of active estrogens can have the ability to affect the etiology of this disease.

After a period that may last several years, the tumor becomes hormone-independent by a mechanism which though not yet fully elucidated is under scrutiny. One explanation for the progression towards hormone-independence may be the presence of ER mutants [5,6]. In hormone-dependent cells, the interaction of the hormone with the receptor molecule is the basic step for eliciting a hormone response. As the cancer cell evolves, mutations, deletions and truncations appear in the receptor gene [7–9]; the ER becomes ‘non-functional’ and, despite the estrogen binding, the cell fails to respond to the hormone. Fig. 1 describes the progression of normal mammary cells towards a hormone-independent carcinoma. A ‘non-functional’ ER might explain why 35–40% of patients with ER-positive tumors fail to respond to anti-estrogen therapy [10,11]. The remaining 5% of breast cancers, denoted BRCA-1, are considered hereditary. The gene was localized on chromosome 17q21 [12,13], but its characterization and use as a marker are still a matter of great controversy (for a review, see Ref. [14]).

Fig. 1. Evolutive transformation of the breast cell from normal to carcinogen. ER+: estrogen receptor positive (detectable and functional); ER mutants: estrogen receptor detectable but non-functional; ER – : estrogen receptor negative (not detectable).

Fig. 2. Enzymatic mechanism involved in the formation and transformation of estrogens in human breast cancer. The sulfatase pathway (A) is quantitatively 100–500 times higher than that of the aromatase pathway (B). $17\beta$-HSD-1 = $17\beta$-hydroxysteroid dehydrogenase type 1.
The majority of breast cancers occur during the postmenopausal period when the ovaries have ceased to be functional. Despite the low levels of circulating estrogens, the tissue concentrations of estrone (E1), estradiol (E2) and their sulfates (E1S, E2S) are several times higher than those found in the plasma or in the area of the breast considered as normal tissue, suggesting a specific tumoral biosynthesis and accumulation of these hormones [15–19].

Several factors could be implicated in this process, including higher uptake of steroids from plasma and local formation of the potent E2 by the breast cancer tissue itself. This information extends the concept of 'intracrinology' where a hormone can have its biological response in the same organ where it is produced.

There is substantial information that mammary cancer tissue contains all the enzymes responsible for the local biosynthesis of E2 from circulating precursors. Two principal pathways are implicated in the last steps of E2 formation in breast cancer tissues: the ‘aromatase pathway’ which transforms androgens into estrogens [20–22] and the ‘sulfatase pathway’ which converts E1S into E1 by the estrone sulfatase (EC: 3.1.6.1) [23–27]. The final step of steroidogenesis is the conversion of the weak E1 to the potent biologically active E2 by the action of a reductive 17β-hydroxysteroid dehydrogenase type 1 activity (17β-HSD-1, EC: 1.1.1.62) [28–30]. Quantitative evaluation indicates that in human breast tumor E1S ‘via sulfatase’ is a much more likely precursor for E2 than is androgens ‘via aromatase’ [17,31].

It is also well established that steroid sulfotransferases (ST), which convert estrogens into their sulfates, are also present in breast cancer tissues [32,33]. Fig. 2 gives a

<table>
<thead>
<tr>
<th>Patients</th>
<th>Estone</th>
<th>Estradiol</th>
<th>Estone sulfate</th>
<th>Authors</th>
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<td>Normal</td>
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<td>2.78 ± 0.22</td>
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<tr>
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<tr>
<td>Mid-cycle</td>
<td>0.55 ± 0.18</td>
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<td>0.88 ± 0.36</td>
<td>0.84 ± 0.35</td>
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<td>1.14 ± 0.18</td>
<td>0.55 ± 0.14</td>
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<td>0.28 ± 0.05</td>
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<td>0.14 ± 0.03</td>
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<td>1.30 ± 0.40</td>
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<td>0.84 ± 0.49</td>
<td>Noel et al., 1981 [49]</td>
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<td>0.25 – 2.62</td>
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<td>0.13 ± 0.01</td>
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<td>0.09 ± 0.01</td>
<td>1.35 ± 0.23</td>
<td>Samojlik et al., 1982 [38]</td>
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<td></td>
<td>0.10 ± 0.04</td>
<td>0.12 ± 0.05</td>
<td>0.05 ± 0.20</td>
<td>0.05 ± 0.02</td>
<td>0.87 ± 0.57</td>
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<td>0.15 ± 0.12</td>
<td>1.50 ± 1.04</td>
<td>1.91 ± 1.06</td>
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<tr>
<td>56–65 years old</td>
<td>–</td>
<td>0.088 ± 0.025</td>
<td>0.073 ± 0.016</td>
<td>0.77 ± 0.021</td>
<td>1.46 ± 0.43</td>
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<td>66–80 years old</td>
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<td>0.082 ± 0.023</td>
<td>0.81 ± 0.22</td>
<td>1.77 ± 0.53</td>
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<td>0.19 – 1.36</td>
<td>0.25 – 4.95</td>
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<td>0.025</td>
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<td>0.12 ± 0.04</td>
<td>0.17 ± 0.05</td>
<td>0.04 ± 0.02</td>
<td>0.06 ± 0.03</td>
<td>0.52 ± 0.13</td>
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**Table 1**

Plasma estrogen concentrations in normal subjects and in breast cancer patients (in pmol/ml)

...
general view of estrogen formation and transformation in human breast cancer.

This review summarizes the recent information concerning the enzymes and their control, involved in the formation and transformation of estrogen in breast cancer.

2. Estrogen concentrations in normal and in breast cancer patients

2.1. In the plasma

Estrogen sulfates are quantitatively one of the most important forms of circulating estrogens. High concentrations of these conjugates are found in the fetal and maternal compartments of humans and several animal species (for a review, see Ref. [34]). During the menstrual cycle [35,36] and in postmenopausal women [37,38] estrone sulfate (E1S) levels are 5–10 times those of unconjugated estrogens (estrone, estradiol, and estriol).

Most authors agree that the plasma levels of unconjugates E1 and E2 are similar in normal women and in breast cancer patients both before and after the menopause (Table 1). However, the E1S level is significantly higher in the follicular phase of premenopausal breast cancer patients than in normal women [17] and in 56- to 80-year-old postmenopausal patients, but not in those aged 48–55 years [39].

2.2. In the breast tissue

Table 2 gives the concentrations of unconjugated E1, E2 and their sulfates, E1S and E2S in breast tumors. In postmenopausal patients, tumor estrogen levels, particularly those of E2 and E1S, are high. E1S can reach 3.35 ± 1.85 pmol/g tissue, that is a level 7 to 11 times that in plasma (ratio = g tissue/ml plasma) [17]. In contrast, in premenopausal patients, tumor E1S levels are two to four times lower than in plasma. Both before and after the menopause, unconjugated estrogen (E1 and E2) levels are comparable. The results obtained for unconjugated estrogen concentrations by most teams are in good agreement [17]. Table 3 details the tissue/plasma ratios of E1, E2, E1S and E2S in pre- and postmenopausal patients with breast cancer [17].

3. Hydroxylated metabolic pathways of estrogens in breast cancer

3.1. 2-Hydroxy derivatives

The conversion of E1 and E2 to C2-hydroxy derivatives in breast cancer tissues and the subsequent formation of catechol O-methyl estrogen by the action of an O-methyltransferase is well documented [57–59]. This transformation is indicated in Fig. 3.

It is interesting that 2-methoxy estradiol can inhibit the proliferation of breast cancer cells [60,61]. As this antiproliferative effect can be obtained in negative ER cell lines, it is suggested that the biological response of 2-methoxy estradiol is mediated by another pathway, that of the classical ER. This assumption is confirmed by the fact that the binding affinity to ER is only 0.1% compared with estradiol [62]. Zhu and Conney [59] suggest that 2-methoxy estradiol can have antitumorigenic and anti-angiogenic effects which can protect estrogen-induced cancer in target organs. It was demonstrated that the activity of 2-methoxy estradiol is mediated independently of estrogen receptor α or β [63].
3.2. 4- and 16-Hydroxy derivatives

4-Hydroxy estrone and 4-hydroxy estradiol possess estrogenic properties and exert a stimulatory effect on the growth of MCF-7 breast cancer cells [64,65]. Elevated 4-hydroxy enzyme activity was found in human breast cancer specimens [66]. Whether 4-hydroxy estrogens are involved in human carcinogenesis is still unclear, but it is interesting to note that high concentrations of 4-hydroxy estrogens are present in human breast tumors [67].

16α-Hydroxy estrone has estrogenic activity which, based on the increased uterine weight of ovariectomized rats, is more potent than that of estradiol itself [68]. It was suggested that 16α-hydroxy estrone could be implicated in carcinogenesis, for instance a comparison of the E2 metabolism of murine mammary epithelial cells revealed that 16α-hydroxylation was significantly elevated in high-risk animals [69,70]. In relation to the proliferative effect of 16α-hydroxy estrone, Lewis et al. [71] showed in MCF-7 breast cancer cells that this estrogen is capable of accelerating cell cycle kinetics and stimulating the expression of cell cycle regulatory proteins. Fig. 3 schematizes the 2–4- and 16α-hydroxylation of estrogens in breast cancer.

4. Sulfatase activity and its control in breast cancer

For many years endocrine therapy in breast cancer has mainly utilized anti-estrogens, which block the estrogen receptor. Treatment with the anti-estrogen tamoxifen (Nolvadex®: tamoxifen citrate) to millions of women with breast cancer has had a significantly beneficial effect resulting in both freedom from symptoms of the disease and reduction in mortality.

More recently, another endocrine therapy has been explored using different anti-enzyme agents involved in the biosynthesis of E2 to inhibit the tissue concentration and production of this hormone. At present, the positive effect of anti-aromatase compounds on the benefit in breast cancer patients is well documented [72,73]. However, as in human breast cancer, E1S is quantitatively the most important precursor of E2 [17,31], new possibilities can be opened to block the E2 originated through this conjugate via the “sulfatase pathway”.

Human estrone sulfatase (EC 3.1.6.1) is a member of an evolutionally conserved protein family in a group of hydrolytic enzymes (human arylsulfatase family: at least six members; classes A, B, C, D, E, and F) and is ubiquitously expressed in mammalian tissues and target organs (e.g. liver, endometrium, ovaries, bone, brain, prostate, white blood cells, adipocytes) but it is particularly prevalent in the placenta and breast carcinoma tissue [74–76].

In recent years, the possible inhibitory effect of estrone sulfatase in breast cancer was explored with a great number of compounds including: anti-estrogens, progestins, tibolone, estradiol, as well as a series of steroidal and non-steroidal substances.

4.1. Inhibition by anti-estrogens

The anti-estrogen tamoxifen and its more important metabolite 4-hydroxytamoxifen, as well as ICI 164,384, have been reported to be inhibitors of sulfatase activity, probably through a non-competitive mechanism [77–80].
4.2. Inhibition by progestins

Various progesterone derivatives (e.g. medrogestone), retro-progesterone derivatives (e.g. dydrogesterone), 19-nortestosterone derivatives (e.g. norethisterone, norelgestromin), 17α-hydroxy-nor-progesterone derivatives (e.g. nomegestrol acetate), 19-norprogesterone derivatives (e.g. promegestone) provoke a significant decrease of estradiol formation when physiological concentrations of estrone sulfate are incubated with breast cancer cells (MCF-7 and T-47D) [81–87]. Fig. 4 provides a comparative study of the inhibitory effect of different progestins on the conversion of E1S to E2 in the T-47D hormone-dependent breast cancer cells.

4.3. Effect of tibolone and its metabolites

In another series of studies, the effect of tibolone on the estrone sulfatase activity was explored. Tibolone (Org OD-14, active substance of Livial®) is a synthetic steroid with a 19-nortestosterone derivative structure. This compound has a tissue-specific action with weak estrogenic, progestagenic and androgenic properties and is extensively used to prevent climacteric symptoms and postmenopausal bone loss. Tibolone and its metabolites Org 4094, Org 30126 (3α and 3β hydroxy derivatives) and its 4-en isomer (Org OM-38) are potent sulfatase inhibitors at low concentrations in hormone-dependent breast cancer cells [88] (Fig. 5).

Using the total breast cancer tissues, it was observed recently that tibolone and its two hydroxy metabolites can also inhibit the estrone sulfatase activity [89].

4.4. Inhibition by steroidal compounds

Estrone-3-O-sulfamate (EMATE) is a potent synthesized sulfatase inhibitor [90]: at a concentration of 10⁻⁷ M the estrone sulfatase activity in MCF-7 cells is inhibited by 99% [91,92]. Unfortunately, the potent estrogenic activity of this compound precludes its use in clinical applications [93,94]. Estrone phosphate and DHEA-phosphate are also potent inhibitors of estrogen sulfatase activity [95].

In other studies, Boivin et al. [96] and Poirier and Boivin [97] attempted to develop sulfatase inhibitors without reside estrogenic activity by synthesizing a series of E2 derivatives bearing an alkyl, a phenyl, a benzyl substituted or not, or an alkyl amide side chain at position 17α. These authors showed that sulfatase inhibitors act by a reversible mechanism and that the hydrophobic group at the 17α position increased the inhibitory activity, while steric factors contributed to the opposite effect. The most potent inhibitor is a 17α-benzyl substituted E2 derivative with an IC₅₀ value of 22 nM. When these 17α-substituents were added to the 3-O-sulfamate estradiol structure, the combined inhibitory effect was more potent. The IC₅₀ value is 0.15 nM [98].

4.5. Inhibition by non-steroidal compounds

A new interesting family of compounds has been synthesized with a tricyclic coumarin sulfamate structure [99–103]. These non-steroidal sulfatase inhibitors are active in vitro and in vivo, are non-estrogenic and possess, in vitro,
Fig. 5. Comparative effects of tibolone (Org OD14, active substance of Livial®) and of its main metabolites on the inhibition of the estrone sulfate (E1S) conversion to estradiol (E2) in the hormone-dependent T-47D human breast cancer cell line. Preconfluent cells were incubated 24h at 37 °C with 5 × 10⁻⁹ mol/l of [³H]-E1S alone or in the presence of tibolone or its metabolites at the concentration of 5 × 10⁻⁷ mol/l. Results (pmol of E2 formed/mg DNA from E1S) are expressed in percent (%) of control values considered as 100%. The data represent the mean ± S.E. of duplicate determinations of three to five independent experiments. Org OM38: 4-en isomer of tibolone; Org 4094: 3α-hydroxy derivative of tibolone; Org 30126: 3β-hydroxy derivative of tibolone. *P ≤ 0.001 vs. control value; **P ≤ 0.0005 vs. control value. Quoted from Ref. [88].

Fig. 6. Effect of estradiol (E2) on the conversion of estrone sulfate (E1S) to E2 in the MCF-7 human breast cancer cells. The percentage of inhibition was obtained by calculating the ratio [(control – test)/control] × 100. The values are the mean ± S.E. of duplicate determinations of five independent experiments. *P ≤ 0.05 vs. control value; **P ≤ 0.005 vs. control value. Quoted from Ref. [104].
an IC₅₀ value of approximately 1 nM. However, the most potent inhibitor in vivo does not correspond to the better compound in vitro.

4.6. Inhibition of estrone sulfatase activity by estradiol

Recent studies have demonstrated a paradoxical effect of E₂ in MCF-7 and T-47D breast cancer cells in that it can block its own bioformation by inhibiting, in a dose-dependent manner, the conversion of E₁St to E₂ in the range of concentrations from 5 × 10⁻¹⁰ to 5 × 10⁻⁵ M [104] (Fig. 6). Estradiol is a potent inhibitory agent of the estrone sulfatase activity as the IC₅₀ values are 1.84 × 10⁻⁹ and 8.77 × 10⁻¹⁰ M in T-47D and MCF-7 cells, respectively [104].

5. Expression and control of estrone sulfatase mRNA

In breast cancer cells, it was observed that the expression of mRNA sulfatase was correlated with the sulfatase activity [105] (Fig. 7), but little is known about the factors regulating steroid sulfatase gene expression in humans. However, it was demonstrated that the progestin promegestone (R-5020), at concentrations of 5 and 50 μmol/l, can inhibit the expression of estrone sulfatase mRNA levels in the MCF-7 and T-47D hormone-dependent breast cancer cell lines by 25% and 50%, respectively [105,106]. This inhibition is correlated with the reduction of the enzymatic activity [107] (Fig. 8).

Newman et al. [108] observed no effect of the cytokine tumor necrosis factor-α (TNF-α) or of interleukin (IL-6) on mRNA sulfatase expression in MCF-7 cells. However, TNF-α and IL-6 increased steroid sulfatase activity in transfected MCF-7 cells with a sulfatase cDNA lacking promoter and enhancer elements. These results suggest that TNF-α and IL-6 may increase sulfatase activity via post-translational modification of the enzyme or by increasing substrate availability.

An interesting study by Utsumi et al. [109] indicated that the steroid sulfatase mRNA level in breast cancer tissues from 38 patients were significantly increased (1458 ± 2119 attomols/mg RNA) as compared with non-malignant tissues (535 ± 663 attomols/mg RNA).

Miyoshi et al. [110] suggest that the sulfatase mRNA levels can serve as a significant, independent poor prognostic factor only in ER-positive tumors. These authors speculate that the up-regulation of sulfatase mRNA levels leads to a high intratumoral estrogen concentration and thus an enhanced stimulation of tumor growth.

6. Sulfotransferases in normal and carcinomous breast

The sulfotransferase responsible for the sulfation reaction consists of two main families: (A) the membrane-associated STs involved in the sulfation of glycosaminoglycans, glycoproteins, and tyrosines in peptides and proteins, and (B) the cytosolic family of STs responsible for the conjugation of steroids, monoamine neurotransmitters, xenobiotics, and drugs. This last family group has two subfamilies: (a) the phenol ST containing the Phenol-PST-1, the Phenol-PST-2, the Monoamine-M-PST, and the estrogen sulfotransferase (EST) isoforms, and (b) the hydroxysteroid sulfotransferases, which include dehydroepiandrosterone (DHEA-ST) and the “brain sulfotransferase-like” ST2B1a and...
STB1b DHEA-ST, which can also conjugate pregnenolone, as well as many bile acids.

The human estrogen sulfotransferases (hEST) involve two isoforms: the hEST-1, which is expressed in various breast cancer cells (e.g. MCF-7, ZR-75-1, T-47D) and is efficient in catalyzing the sulfation of 2-hydroxy-estrone and 2-hydroxy-estradiol, and the hEST-2, which selectively catalyzes sulfonation of estradiol, estrone, and ethinyl estradiol [33,111,112].

6.1. Normal breast

Wild et al. [113] have observed very high levels of estrone sulfotransferase (EST) in a “normal” breast cell line produced by a Simian Virus (SV) 40, immortalization of breast epithelial cells obtained from reduction mammoplasty (Huma-7). The EST activity in this cell line far exceeded that in either MCF-7 or ZR-75-1 breast cancer cells. In the normal cell after 24 h culture, 50% of the substrate was sulfated compared with less than 10% in the malignant cells. This study was confirmed by Anderson and Howell [114] using two normal breast epithelial cells: the MTSV 1–7 and the MRSV 4–4 produced by SV 40 immortalization cells obtained from human milk [115].

Among the different human STs, only hEST has the affinity for estradiol sulfation in the nanomolar concentration range. Consequently, hEST may be active in altering the levels of unconjugated estrogens in the cell, and thus cellular responsiveness to estrogens, as estrogens in the nanomolar concentration range interact with the ER.

When human mammary epithelial cells (HME) are established initially, they are estrogen-dependent [116]. Studies using immunohistochemical ER, a method more sensitive than the classical biochemical receptor assays, confirm the presence of ER in HME cells [117]. Estrogen-dependent cells with high EST levels grow more slowly than cells with lower levels of EST or no detectable EST. Metabolic evidence indicates that this is due to the ability of EST to render estrogens physiologically inactive via sulfate conjugation [33,118].

6.2. Breast cancer

The presence of sulfotransferases in normal and carcinomous breast is extensively demonstrated [120–122]. However, there are discrepancies concerning sulfotransferase activities: some authors found only PST or HST activity, but not EST, in the hormone-dependent breast cancer cells, as well as in the hormone-independent BT-20 cells and detected no ST activity in MDA-MB-231 or MDA-MB-468 cells; whereas other authors report EST and HST activity in MCF-7 and ZR-75 cells and in mammary tumors. These variations are probably caused by different factors, including cell origin, culture conditions, instability of human EST enzyme, and the condition of the enzyme assays.

Falany and Falany [33] considered that hEST is not detectable in most breast cancer cell lines and suggested...
that the sulfoconjugated activity in the cells is mainly due to the human Phenol-PST, an enzyme that has a higher affinity with the estrogens at micromolar than at nanomolar concentrations. hP-PST has an affinity for estrogen sulfation about 300-fold lower than that of hEST [119,123].

To explore the difference in EST content between normal human mammary epithelial and breast cancer cells, and their correlation with cellular growth, Falany and Falany [33,124] transformed MCF-7 cells with an EST expression vector, and observed that after incubation of 20 nM of E2, sulfation occurs more rapidly with MCF-7 cells transformed with EST than with the control cells, thereby rendering E2 physiologically inactive. EST/MCF-7 cells require a higher concentration of E2 to stimulate growth than do control MCF-7 cells, as EST inactivates E2 via sulfatation, consequently rendering it incapable of binding to the ER and inhibiting the process of cell growth.

In conclusion, knowledge of the expression and regulation of the different sulfotransferases is of capital importance in understanding the changes in the normal breast cell during tumorigenesis, as well as hormonal involvement in this mechanism.

6.3. Control of sulfotransferase activities in breast cancer

As sulfoconjugates are not biologically active, the control of the formation of these conjugates in breast cells represents an important mechanism to modulate the biological action of estradiol in this tissue.

Comparative studies on the formation of estrogen sulfates after incubation of estrone with the hormone-dependent (MCF-7, T47D) and hormone-independent (MDA-MB-231) breast cancer cells show significantly higher sulfotransferases in the former [125].

6.3.1. Effect of medrogestone and other progestins

Medrogestone is a synthetic pregnane derivative used in the treatment of pathological deficiency of the natural progestosterone. This compound produces secretory activity in the estrogen-primed uterus, is thermogenic and acts as an anti-estrogen and antigonadotropin. Concerning the effect of medrogestone on sulfotransferase activity in MCF-7 and T47-D breast cancer cells, it was observed that this progestin has a biphasic effect: at a low concentration (5 × 10^{-8} mol/l) it stimulates the formation of estrogen sulfates in both cell lines, whereas at a high concentration (5 × 10^{-5} mol/l) the sulfotransferase activity is not modified in MCF-7 cells or is inhibited in T-47D cells (Fig. 9) [126]. Fig. 10 gives a comparative study on the effect of medrogestone and other progestins (e.g. nomegestrol acetate, promegestone (R-5020)) on sulfotransferase activity in T-47D breast cancer cells.

6.3.2. Effect of tibolone and its metabolites

Tibolone (the active substance in Livial), is a 19-nortestosterone derivative with estrogenic, androgenic and progestagenic properties used to prevent climacteric symptoms and postmenopausal bone loss [127,128].

In a series of studies, the effects on sulfotransferase activity of tibolone and its metabolites: 3α-hydroxy (Org 4094), 3β-hydroxy (Org 30126) and the 4-ene isomer (Org OM-38) were explored in MCF-7 and T-47D breast cancer cells.

![Fig. 9. Effects of Medrogestone (Prothil®) on the conversion of estrone (E1) to estrogen sulfates in the hormone-dependent MCF-7 and T47D human breast cancer cell lines. Preconfluent cells were incubated 24 h at 37 °C with 5 × 10^{-9} mol/l of [3H]-E1 alone (control; non-treated cells) or in the presence of medrogestone. Results (pmol of ES formed/mg DNA) are expressed in percent (%) of control values considered as 100%. The data are the mean ± S.E. of duplicate determinations of three independent experiments. *P ≤ 0.5 vs. control value (non-treated cells); **P ≤ 0.01 vs. control value (non-treated cells). Quoted from Ref. [126].](image-url)
cells. These compounds also provoke a dual effect on sulfotransferase activity: stimulatory at low doses (5 \(\times\) 10^{-8} mol/l), whereas an inhibition of this activity is observed at higher doses (5 \(\times\) 10^{-5} mol/l). It is to be remarked that the 3β-hydroxy derivative is the most potent compound in the stimulatory effect of ST [129].

Estrogen sulfates are found exclusively in the culture medium, indicating that the enzyme acts near the plasma membrane and secretes ES in the culture medium. For the hormone-independent MDA-MB 231 cells, the EST activity is very low and none of the different estrogens tested had a significant effect on this activity. This difference between hormone-dependent and hormone-independent cells could reflect the presence of various isoforms of EST or several other STs (such as phenol-ST or hydroxy-ST) with different kinetic properties, which are also able to conjugate estrogens but at micromolar concentrations [123,130]. It is probable that the different alterations of cellular metabolism in the cells can also affect the production of the cofactor PAPS which contributes to the regulation of sulfotransferases activities.

7. Sulfotransferase expression and its control in breast cancer

The placental hEST-1 gene consists of nine exons and eight introns and is approximately 7.7 kb in length; the expressed enzyme was able to transform estrone to estrone sulfate at nanomolar concentrations [131]. It was demonstrated that a single gene, assigned to chromosome 16, can transcribe at the same time brain phenol sulfotransferase (PST or HAST), M-PST, and human placental EST 1 mRNA by alternate exon 1b and exon 1b promoters, respectively [132].

Qian et al. [118] demonstrated that the restoration of EST expression in MCF-7 cells by cDNA transfection could significantly attenuate the response on both gene activity and DNA synthesis, and cell numbers were used as markers of estrogen-stimulated cell growth and proliferation. These authors suggested that loss or downregulation of estrogen sulfotransferase may enhance the growth-stimulating effect of estrogens and contribute to the process of tumor initiation.

Using reverse transcriptase-polymerase chain reaction amplification, the expression of estrogen sulfotransferase mRNA was detected in the hormone-dependent MCF-7 and T-47D, as well as in the hormone-independent MDA-MB-231 and MDA-MB-468, human breast cancer cells. An interesting correlation of the relative sulfotransferase activity and the human estrogen sulfotransferase type 1 mRNA expression was found in the various breast cancer cells studied [133] (Fig. 11).

A study on the effects of the progestin promegestone (R-5020) on the activity of type 1 hEST and its mRNA in the MCF-7 and T-47D cells shows that at low doses of R-5020...
there is a significant increase in the levels of mRNA hEST in these breast cancer cell lines, which correlates with hEST enzyme activity. However, at high doses of this progestin an inhibitory effect is observed in hEST and its mRNA [133] (Fig. 12).

8. Hypothetical correlation of proliferation of the breast cancer cell and sulfotransferase activity

Maximal epithelial mitosis of the normal breast cell is found between 22 and 26 days of the cycle, which corre-
sponds to the high levels of estradiol and progesterone [134]. During pregnancy, it is suggested that the elevated values of circulating progesterone are responsible for the induction of lobular-alveolar development, to prepare the breast for lactation [135,136]. The data on the effect of progesterone on breast epithelial proliferation are contradictory. It has been found that progesterone can increase DNA synthesis in normal breast epithelium in organ culture [137]. Using normal epithelial cells of human breast, it was demonstrated that the progestin promegestone can decrease cell proliferation [138,139]. These authors also found that progestins can inhibit the proliferative effect provoked by estradiol, whereas McManus and Welsch [140] and Longman and Buehring [141] demonstrated no effect.

The proliferative effect of progestins using various isolated breast cancer models: cell lines, organ culture, or transplantation of breast cancer cells in nude mice, is contradictory as it was reported that these compounds can either inhibit [142–145], stimulate [146–148], or have no effect [149].

It was demonstrated that in normal breast cells the estrogen hEST, which is active at nanomolar concentrations of estradiol, mainly present to form estradiol sulfate (E2S) and consequently to block the proliferative effect of estradiol as E2S, is biologically inactive. However, in the breast cancer cells the phenol sulfotransferase (P-ST), which is active at micromolar concentrations of E2 (see Schemes 1 and 2), is present and the hEST is not present [33,117,123]. As the progestins nomegestrol acetate or medrogestone can stimulate hEST in breast cancer cells, and as these compounds can block the proliferation in breast cancer cells, it is suggested that the antiproliferative effect of nomegestrol acetate or medrogestone is correlated with the stimulatory effect of hEST in the hormone-dependent breast cancer cells (Scheme 3). More information on the correlation of the proliferative effect and hEST on breast cancer cells of various progestins or other substances is needed to verify this hypothesis.

9. 17β-Hydroxysteroid dehydrogenase and its control in breast cancer

The last step of biosynthesis of the potent biologically active estrogen, estradiol, in target tissues is the conversion of estrone to estradiol by the reductive 17β-HSD 1 (EC 1.1.1.62) activity.

17β-HSD is a widely distributed enzyme in mammalian tissues, which is implicated in the interconversion of the inactive 17β-keto-<→ into active 17β-hydroxy in sex steroid hormones (estrogens and androgens). However, some types of 17β-HSD may metabolize further substrates such as bile acids, alcohols, fatty acids and retinols. 17β-HSD belongs to a superfamily of enzymes (to date up to 11 different isoforms are recognized).

9.1. Normal breast

In normal breast tissue, it was observed that the oxidative 17β-HSD activity (E2 to E1) is the preferential direction and that this activity is more intense during the secretory phase.
of the menstrual cycle [150]; 17β-HSD types 1 and 2 mRNAs were both expressed in the glandular epithelium. In HME cell line, mRNAs for 17β-HSD types 1, 2, and 4 were detected, but only oxidative 17β-HSD activity was present and it was suggested that this activity is due to 17β-HSD type 2 [151].

Using epithelial cells of normal breast, it was observed that the progestin promegestone (R-5020) can increase the 17β-HSD activity in the oxidative (E2 to E1) direction; this stimulatory effect of the progestins depends on preliminary sensitization by the estrogens [138,152].

9.2. Breast cancer

In breast tumors, in vivo and in vitro studies indicate that the preferential conversion is the reduction of E2 to E1. The 17β-HSD type 1 is located in the cytoplasm of malignant epithelial cells of breast tumors [153]. However, it was observed that the orientation of the enzymatic activity (oxidative or reductive) in breast cancer is also greatly dependent on the local, metabolic or experimental conditions, including: the nature and concentration of the cofactors (e.g. NADPH or NADP) and of substrate, pH, subcellular localization of enzymes. In vitro studies using human tumor homogenates indicated that the predominant 17β-HSD activity was oxidative rather than reductive [28]. However, in vivo studies, after isotopic infusion of estrogens to postmenopausal breast cancer patients, have shown that the reductive direction is greater than the oxidative [29].

In hormone-dependent breast cancer cell lines (MCF-7, T-47D, R-27, ZR-75-1) 17β-HSD type 1 was the predominant reductive isoform, but type 2 and 4 isoforms with oxidative activities (formation of E1) were also detected [30,153–155]. It was demonstrated that in intact cells, when the physiological conditions are more closely protected, the catalytic activity of each type of 17β-HSD is exclusively uni-directional, whereas in cell homogenates the bidirectional orientation prevails, but the physiological direction is favoured [156,157].

In contrast, when breast cancer cells evolve to a hormone-independent status (MDA-MB-231; MDA-MB-436; Hs-578S) they revert to the oxidative (E2 to E1) 17β-HSD activity as their preferential enzymatic orientation [30]. This observation suggests that there is a change in 17β-HSD phenotype in neoplastic cells and that the tumoral process of the breast is accompanied by a modification of estrogen metabolism [158].

Fournier et al. [159] have postulated that 17β-HSD might be a marker for hormone-dependent breast cancer. In more recent studies, Suzuki et al. [160,161] observed that 17β-HSD type 1 was immunolocalized in carcinoma cells in 68 out of 111 invasive ductal carcinoma cases, while 17β-HSD type 2, which catalyzes the conversion of E2 to E1, was not detected in any of these cases. These authors show a significant correlation between 17β-HSD type 1 and ER and PR expression, which is in agreement with the data of Sasano et al. [162] who showed also that 17β-HSD type 2 is greatly expressed in endometrial carcinoma. Ariga et al. [163] also found that 17β-HSD type 1 is preferentially localized in breast tumors and 17β-HSD type 2 in normal breast, but there is no significant correlation between ER and 17β-HSD type 1. Recent quantitative real-time PCR data seem to indicate that 17β-HSD type 1 mRNA expression levels were significantly higher in postmenopausal than in premenopausal breast cancer patients [110].

9.3. Control of 17β-hydroxysteroid dehydrogenase activity in the breast

9.3.1. Control by progestins

Breast tumors from postmenopausal patients receiving lynestrenol display higher oxidative 17β-HSD activity than tumors from untreated patients. The activity depends on the ER or PR status of the tumor [159].

Progestins can induce 17β-HSD type 1 activity with an increase in both the 1.3 kb mRNA species and enzyme protein in hormone-dependent T-47D breast cancer cells [153,164,165]. Org 2058 increases the oxidative direction in T-47D cells only [153]. Coldham and James [166] showed that the progestin medroxyprogesterone acetate (MPA) stimulates the reductive (E1 to E2) activity of MCF-7 cells when phenol red was excluded from the tissue culture media. The authors suggested that this could be the way in which progestins increase cell proliferation in vivo. On the other hand, Couture et al. [154] observed that in the treatment of hormone-dependent ZR-75-1 breast cancer cells with MPA, the oxidative (E2 to E1) direction is predominant; this effect seems to implicate the androgen receptor. Other progestins, such as progesterone, levonorgestrel, and norethisterone, increase both the oxidative and reductive 17β-HSD activity in MCF-7 cells [167], whereas promegestone (R-5020) has no significant effect on the reductive activity of 17β-HSD [30] but can increase the oxidative (E2 to E1) activity in T-47D cells [168].Nomegestrol acetate has an inhibitory effect on the 17β-HSD enzyme in T-47D cells (35% and 81% inhibition at 5×10⁻⁷ and 5×10⁻⁶ M, respectively) but no significant effect was found in MCF-7 cells, except at 5×10⁻⁵ M [83]. Medrogestone (Prothil®), a synthetic pregnane derivative of progesterone, significantly decreases the reductive 17β-HSD type 1 activity in MCF-7 and T-47D breast cancer cells. The inhibitory effect is dose-dependent and is more intense, even at low doses, in the T-47D cell line than in the MCF-7 cells; the IC₅₀ values, which correspond to the 50% inhibition of the conversion of E₁ to E₂, are 0.45 and 17.36 μM, respectively [125] (Fig. 13).

9.3.2. Control by tibolone and its metabolites

Tibolone (Org OD14), a 19-nortestosterone derivative with tissue-specific estrogenic, androgenic or progestagenic properties, significantly decreases the reductive activity of
17β-HSD in hormone-dependent T-47D and MCF-7 breast cancer cells [170]. This inhibitory effect is dose-dependent and was significant at a concentration of $5 \times 10^{-7}$ M. The $3\alpha$-OH and $3\beta$-OH metabolites of tibolone (Org 4094 and Org 30126, respectively) also show a similar inhibitory effect. The 4-en isomer of tibolone (Org OM38) shows an inhibitory effect only at the concentration of $5 \times 10^{-6}$ M; the IC50 values in T-47D cells are respectively: 1.44, 2.03, 4.83, and 35.25 nM for Org 30126, tibolone, Org 4094, and Org OM38 [169].

9.3.3. Control by anti-estrogens and other compounds

The anti-estrogen ICI 164,384 can inhibit by competition the enzyme 17β-HSD in human breast tumors (IC50 value: 890 nM) [80]. However, in our laboratory we found that ICI 164,384 at $5 \times 10^{-6}$ M inhibits by 53% the conversion of E1 to E2 in T-47D cells [30].

Various potential irreversible or reversible inhibitors of 17β-HSD type 1 have been synthesized (e.g., bromoacetoxyl or alkylamide derivatives of E2 and of progesterone) [171–173]. Thus, for example, the compound 16α-(bromoalkylamide) derivative of E2 inhibits the 17β-HSD type 1 in human placenta with an IC50 value of 10.6 μM [173]. Sawicki et al. [173] obtained 77% inhibition of 17β-HSD type 1 activity with equilin, a component used in estrogen replacement therapy (ERT), at the concentration of 1 μM.

In a recent interesting study, Gunnarsson et al. [174] observed that the expression of 17β-hydroxysteroid dehydrogenase type I or type II can correlate to recurrence-free survival (RFS) of patients with breast cancer; low levels of mRNA 17β-HSD type II was related to decreased RFS.

10. Aromatases and anti-aromatase

The aromatase cytochrome P450 catalyzes aromatization of androgens to estrogens; biochemical and immunocytochemical studies have revealed the presence of this enzyme in the adipose stromal cells of breast cancer tissues. Although levels of aromatase activity are relatively low in the breast, this local production of estrogens ‘on site’ can contribute to the pathogenesis of estrogen-dependent breast cancers.

Aromatase inhibition by anti-aromatase agents is largely developed with very positive results in the treatment of patients with breast cancer. These inhibitors include steroidal and non-steroidal compounds. The most useful are: aminogluthethimide, 4-hydroxy-androstenedione (Formestane; Lentaron®), Vorosole, Letrozole (Femara®), Anastrozole (Arimidex®), Examestane (Aromasin®). A series of reviews has been published recently on the biological effects and the therapeutic applications of these anti-aromatases [22,175,176].

11. Conclusions

One of the possible ways of blocking the estradiol effect in breast cancer is the use of anti-estrogens, which act by binding to the ER. More than 15 years’ experience have
strone sulfate. Estrone sulfatase. ANDR.: androgens; E1: estrone; E2: estradiol; E1S: estrone sulfatase; SEEM-II the 17β-hydroxysteroid dehydrogenase (17β-HSD) which are involved in estradiol biosynthesis in breast cancer tissues. At present, anti-aromatases are extensively used in breast cancer treatment with positive benefits. However, estrone sulfatase is quantitatively the most important pathway in estradiol bioformation in breast cancer tissue. Very interesting data were obtained concerning the inhibitory activity of various progestins (promegestone, nomegestrol acetate, medrogestone, dydrogesterone, norelgestromin), as well as tibolone and its metabolites, on estrone sulfatase, as well as on 17β-hydroxysteroid dehydrogenase, enzymes involved in the other pathway of estradiol formation in breast cancer cells.

Recent data also show that some progestins (promegestone, nomegestrol acetate, medrogestone) as well as tibolone can stimulate sulfotransferase activity in hormone-dependent breast cancer cells. This is an important point in the physiopathology of this disease, as it is well known that estrogen sulfates are biologically inactive.

The fact that estradiol (E2) can block its own bioformation in the breast cancer cell provides another aspect of this very complex mechanism in breast cancerization which, in addition to growth factors, oncogenes, proto-oncogenes and other factors, needs extensive additional information to be clarified. The paradoxical effect of E2 could be related to ERT, a treatment that has been observed to have either no effect or to slightly increase breast cancer incidence [177] but significantly decrease mortality [178–182].

For these inhibitory or stimulatory effects on the control of the enzymes involved in the formation and transformation of estrogens in breast cancer, we have proposed the concept of selective estrogen enzyme modulators (SEEM), which is schematically represented in Fig. 14.

The exploration of various progestins and other substances in trials with breast cancer patients, showing an inhibitory effect on sulfatases and 17β-hydroxysteroid dehydrogenase and a stimulatory effect on sulfotransferases will, in combination with anti-aromatase agents, provide new possibilities in the treatment of this disease.

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