Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochimica et Biophysica Acta 1654 (2004) 123–143
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.bba-direct.com

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

Received 21 January 2004; accepted 12 March 2004

Available online 15 April 2004

Abstract

It is well established that increased exposure to estradiol (E_2) 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 E_2 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 E_2 from circulating precursors. Two principal pathways are implicated in the last steps of E_2 formation in breast cancer tissues: the 'aromatase pathway' which transforms androgens into estrogens, and the 'sulfatase pathway' which converts estrone sulfate (E_1S) into E_1 by the estrone-sulfatase. The final step of steroidogenesis is the conversion of the weak E_1 to the potent biologically active E_2 by the action of a reductive 17β-hydroxysteroid dehydrogenase type 1 activity (17β-HSD-1). Quantitative evaluation indicates that in human breast tumor E_1S 'via sulfatase' is a much more likely precursor for E_2 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 E_2 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, nomegestrol 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 E_2, will provide a new possibility in the treatment of this disease.

Keywords: Progestin; 17β-Hydroxysteroid dehydrogenase; Estradiol

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),

* Tel.: +33-1-4542-4121/4539-9109; fax: +33-1-4542-6121.
E-mail address: jorge.pasqualini@wanadoo.fr (J.R. Pasqualini).

0304-419X/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbcan.2004.03.001

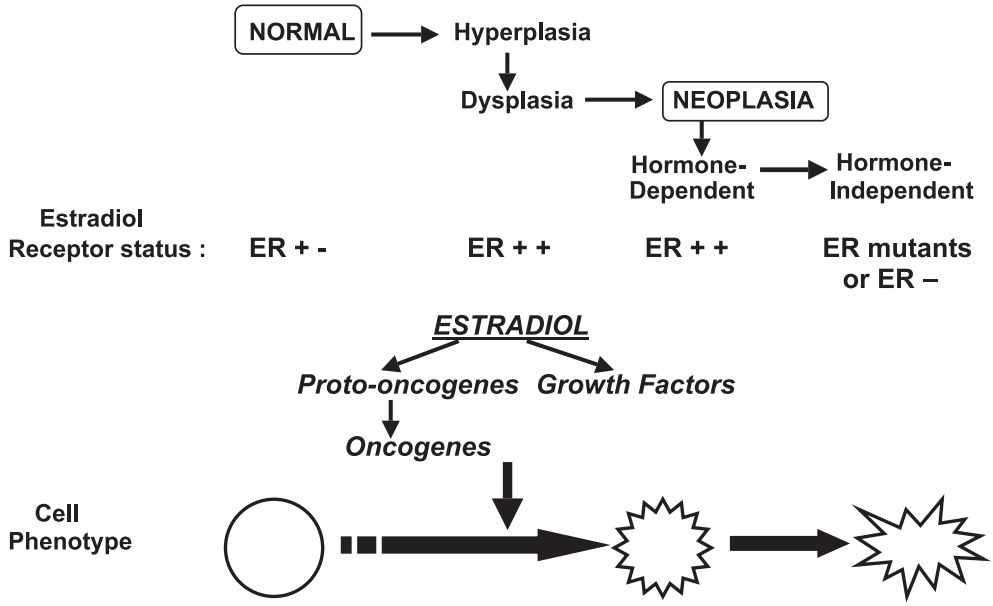


Fig. 1. Evolutive transformation of the breast cell from normal to carcinogen. ER+: estrogen receptor positive (detectable and functional); ER mutants: estrogen receptor detectable but non-functional; ER − : estrogen receptor negative (not detectable).

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]).

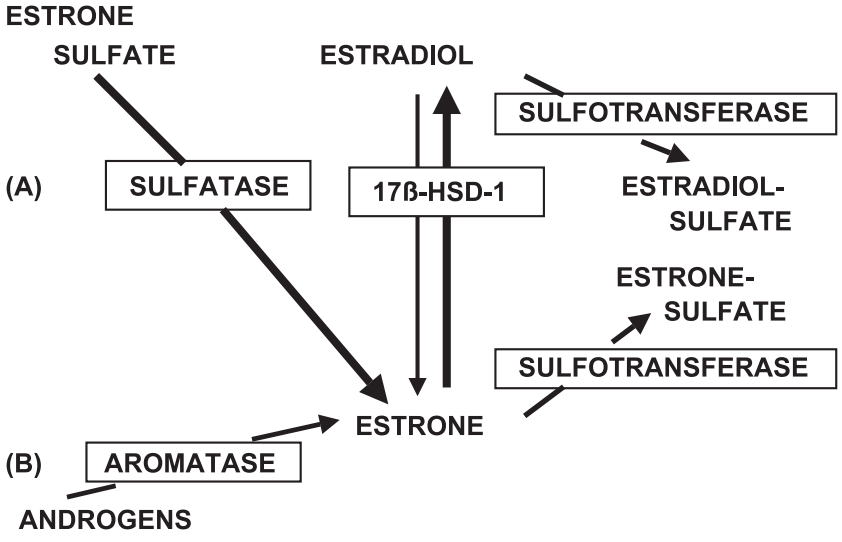


Fig. 2. Enzymatic mechanism involved in the formation and transformation of estrogens in human breast cancer. The sulfatase pathway (A) is quantitatively 100–500 times higher than that of the aromatase pathway (B). 17β-HSD-1 = 17β-hydroxysteroid dehydrogenase type 1.

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 tissular concentrations of estrone (E_1), estradiol (E_2) and their sulfates (E_1S, E_2S) 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 E_2 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 E_2 from circulating precursors. Two principal pathways are implicated in the last steps of E_2 formation in breast cancer tissues: the 'aromatase pathway' which transforms androgens into estrogens [20–22] and the 'sulfatase pathway' which converts E_1S into E_1 by the estrone sulfatase (EC: 3.1.6.1) [23–27]. The final step of steroidogenesis is the conversion of the weak E_1 to the potent biologically active E_2 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 E_1S 'via sulfatase' is a much more likely precursor for E_2 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 1
Plasma estrogen concentrations in normal subjects and in breast cancer patients (in pmol/ml)

Patients	Estrone		Estradiol		Estrone sulfate		Authors	Reference
	Normal	Breast cancer	Normal	Breast cancer	Normal	Breast cancer		
Premenopausal								
Follicular phase	–	–	–	–	1.33 ± 0.78	–	Loriaux et al., 1971	[41]
Luteal phase	–	–	–	–	2.55 ± 1.29	–	Loriaux et al., 1971	[41]
Follicular phase	–	–	–	–	2.78 ± 0.22	–	Wright et al., 1978	[42]
Luteal phase	–	–	–	–	5.17 ± 0.45	–	Wright et al., 1978	[42]
Mid-cycle	0.55 ± 0.18	0.77 ± 0.16	0.88 ± 0.36	0.84 ± 0.35	–	–	Drafta et al., 1980	[43]
Late-cycle	0.70 ± 0.20	0.81 ± 0.20	1.14 ± 0.18	0.55 ± 0.14	–	–	Drafta et al., 1980	[43]
Follicular phase	–	–	–	–	1.96 ± 0.35	–	Samojlik et al., 1982	[38]
Mid-cycle	–	–	–	–	6.41 ± 1.81	–	Samojlik et al., 1982	[38]
Luteal phase	–	–	–	–	5.61 ± 1.06	–	Samojlik et al., 1982	[38]
Luteal phase	–	–	–	–	2.54 (0.91–4.45)	–	Hawkins and Oakey, 1974	[35]
Luteal phase	–	–	–	–	7.60 ± 1.05	–	Honjo et al., 1987	[36]
Luteal phase	–	–	–	0.16 ± 0.42	–	–	Stein et al., 1990	[44]
Luteal phase	–	–	–	0.44 ± 0.36	–	–	Blamey et al., 1992	[45]
Luteal phase	–	–	–	0.28 ± 0.05	–	–	Neskovic-Konstantinovic et al., 1994	[46]
Luteal phase	–	0.29 ± 0.10	–	0.36 ± 0.12	–	–	Massobrio et al., 1994	[47]
Follicular phase	0.16 ± 0.04	0.19 ± 0.08	0.25 ± 0.07	0.21 ± 0.13	1.86 ± 0.50	3.64 ± 1.17	Pasqualini et al., 1996	[17]
Luteal phase	0.21 ± 0.04	0.21 ± 0.04	0.35 ± 0.08	0.23 ± 0.06	4.17 ± 0.21	3.97 ± 1.88	Pasqualini et al., 1996	[17]
Postmenopausal								
	–	–	–	–	–	1.35 ± 2.55	Ruder et al., 1972	[48]
	–	–	–	–	0.11 ± 0.92	–	Roberts et al., 1980	[37]
	0.14 ± 0.03	–	–	–	1.30 ± 0.40	–	Towobola et al., 1980	[39]
	–	–	–	–	0.84 ± 0.49 (0.25–2.62)	–	Noel et al., 1981	[49]
	–	–	–	–	1.35 ± 0.23	1.20 ± 0.22	Samojlik et al., 1982	[38]
	–	0.13 ± 0.01	–	0.09 ± 0.01	–	–	Bonney et al., 1983	[28]
	0.10 ± 0.04	0.12 ± 0.05	0.050 ± 0.020	0.05 ± 0.02	0.87 ± 0.57	0.83 ± 0.51	Reed et al., 1983	[50]
48–55 years old	–	–	0.090 ± 0.046	0.15 ± 0.12	1.50 ± 1.04	1.91 ± 1.06	Prost et al., 1984	[40]
56–65 years old	–	–	0.088 ± 0.025	0.073 ± 0.016	0.77 ± 0.021	1.46 ± 0.43	Prost et al., 1984	[40]
66–80 years old	–	–	0.109 ± 0.023	0.082 ± 0.023	0.81 ± 0.22	1.77 ± 0.53	Prost et al., 1984	[40]
	–	–	–	–	0.19–1.36	0.25–4.95	Hawkins et al., 1985	[51]
	–	0.11 ± 0.06	–	0.07 ± 0.03	–	0.92–0.80	Vermeulen et al., 1986	[16]
	–	0.11	–	–	–	0.70	Lonning et al., 1989	[52]
	–	0.13 ± 0.06	–	0.07 ± 0.03	–	–	Massobrio et al., 1994	[47]
	–	–	–	0.025 (0.011–0.055)	–	–	Recchione et al., 1995	[53]
	0.12 ± 0.04	0.17 ± 0.05	0.04 ± 0.02	0.06 ± 0.03	0.52 ± 0.13	0.37 ± 0.19	Pasqualini et al., 1996	[17]

Table 2
Concentrations of unconjugated estrogens and their sulfates in malignant breast tissue (in pmol/g tissue)

Estrone	Estradiol	Estrone sulfate	Estradiol sulfate	Authors	Reference
Premenopausal					
1.04	0.70	–	–	van Landeghem et al., 1985	[54]
1.40 ± 0.08	1.20 ± 0.60	1.27 ± 0.36	0.92 ± 0.27	Pasqualini et al., 1994	[55]
Postmenopausal					
1.14 ± 0.22	1.80 ± 0.29	–	–	Reed et al., 1983	[50]
0.60	0.78	–	–	van Landeghem et al., 1985	[54]
25.18 ± 51.10[a] (0.37–248)	32.72 ± 37.95[a] (1.47–180.50)	14.61 ± 19.77[a] (0.28–97.70)	–	Vermeulen et al., 1986	[16]
0.25	0.60	–	–	Thijssen and Blankenstein, 1989	[56]
1.00 ± 0.15	1.40 ± 0.70	3.35 ± 1.85	1.47 ± 0.11	Pasqualini et al., 1996	[17]
1.06 ± 0.43	1.27 ± 0.59	2.89 ± 1.93	0.97 ± 0.56	Pasqualini et al., 1994	[55]
–	0.169 (0.033–0.775)	–	–	Recchione et al.,1995	[53]

[a] In pmol/g protein.

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 (E_1S) levels are 5–10 times those of unconjugated estrogens (estrone, estradiol, and estriol).

Most authors agree that the plasma levels of unconjugates E_1 and E_2 are similar in normal women and in breast cancer patients both before and after the menopause (Table 1). However, the E_1S 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].

Table 3
Ratio concentrations in the tumor tissue/plasma of estrone, estradiol, and their sulfates in human breast cancer

Patients	Estrone	Estradiol	Estrone sulfate	Estradiol sulfate
Premenopausal	7	5	0.3	2
Postmenopausal	6	23	9.0	3

The ratio corresponds to values obtained with the tissue concentration of each estrogen (pmol/g) divided by the plasma concentration of the respective estrogen (pmol/ml). The data represent the average of 11 to 15 patients. Quoted from Ref. [17].

2.2. In the breast tissue

Table 2 gives the concentrations of unconjugated E_1, E_2 and their sulfates, E_1S and E_2S in breast tumors. In postmenopausal patients, tumor estrogen levels, particularly those of E_2 and E_1S, are high. E_1S 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 E_1S levels are two to four times lower than in plasma. Both before and after the menopause, unconjugated estrogen (E_1 and E_2) 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 E_1, E_2, E_1S and E_2S 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 E_1 and E_2 to C_2-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].

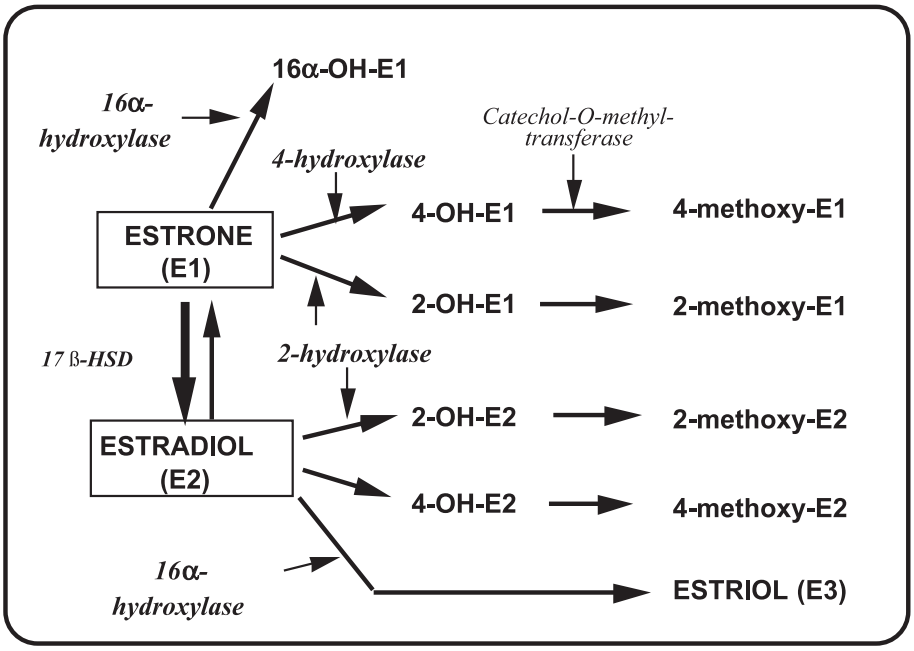


Fig. 3. Hydroxyl pathways of estrogens in human breast cancer. 2-OH-E1: 2-hydroxy-estrone; 2-OH-E2: 2-hydroxy-estradiol; 4-OH-E1: 4-hydroxy-estrone; 4-OH-E2: 4-hydroxy-estradiol; 16α-OH-E1: 16α-hydroxy-estrone.

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 E_2 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 E_2 to inhibit the tissular 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, E_1S is quantitatively the most important precursor of E_2 [17,31], new possibilities can be opened to block the E_2 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 E_1S to E_2 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 in 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 residue estrogenic activity by synthesizing a series of E_2 derivatives bearing an alkyl, a phenyl, a benzyl substituted or not, or an alkyan 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 E_2 derivative with an IC_{50} 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 IC_{50} 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,

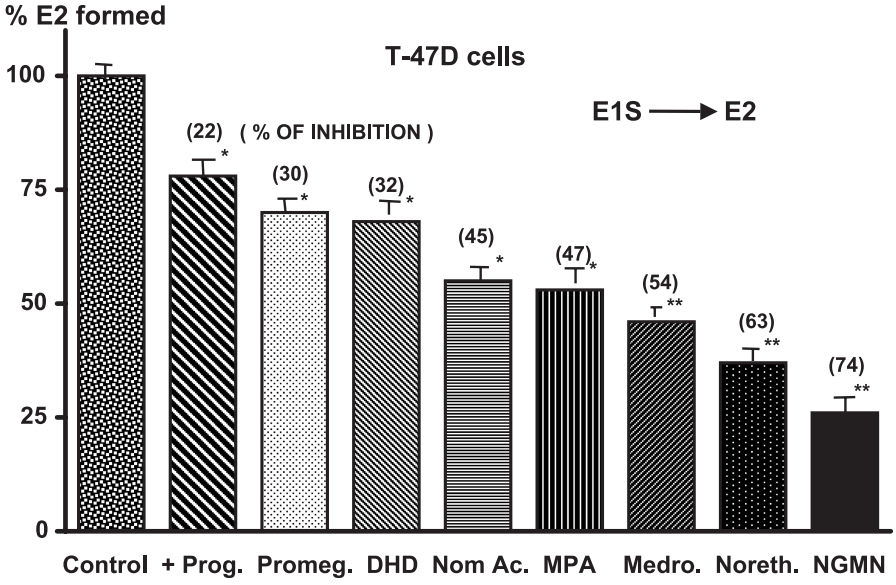


Fig. 4. Comparative effects of various progestins on the inhibition of the estrone sulfate (E_1S) conversion to estradiol (E_2) in the hormone-dependent T-47D human breast cancer cell line. Preconfluent cells were incubated 24 h at 37 °C with a physiological concentration (5×10^{-9} mol/l) of [^{3}H]-estrone sulfate (E_1S) alone or in the presence of progestins at the concentration of 5×10^{-7} mol/l. Results (pmol of E_2 formed/mg DNA from E_1S) are expressed in percent (%) of control value considered as 100%. The data represent the mean ± S.E. of duplicate determinations of three to seven independent experiments. Prog.: progesterone; Promeg.: promegestone; DHD: 20-dihydro-dydrogesterone; Nom.Ac.: nomegestrol acetate; MPA: medroxyprogesterone acetate; Medro.: medrogestone; Noreth.: norethisterone; NGMN: norelgestromin. *$P \leq 0.05$ vs. control value; **$P \leq 0.01$ vs. control value.

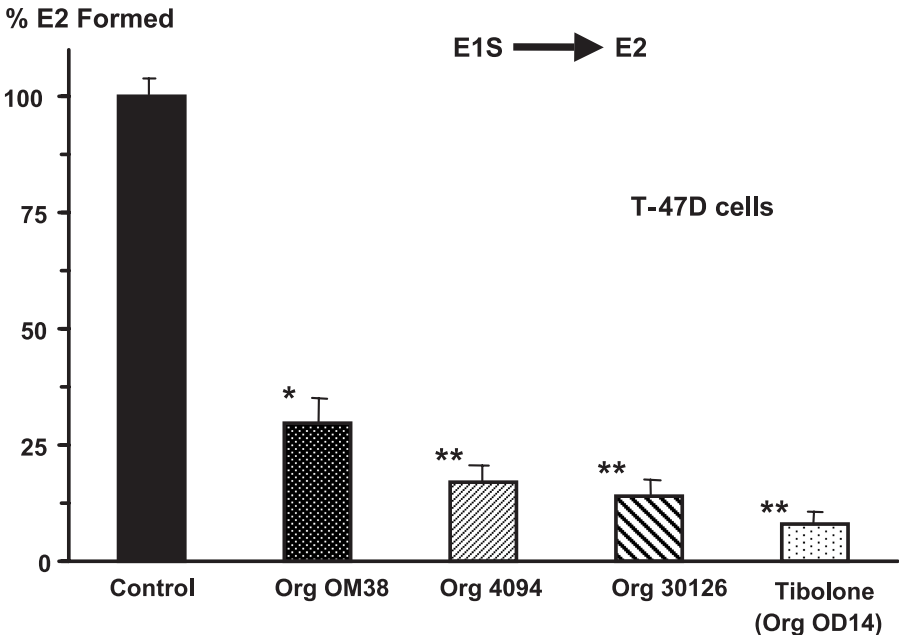


Fig. 5. Comparative effects of tibolone (Org OD14, active substance of Livial®) and of its main metabolites on the inhibition of the estrone sulfate (E_1S) conversion to estradiol (E_2) in the hormone-dependent T-47D human breast cancer cell line. Preconfluent cells were incubated 24h at 37 °C with 5×10^{-9} mol/l of [^{3}H]-E_1S alone or in the presence of tibolone or its metabolites at the concentration of 5×10^{-7} mol/l. Results (pmol of E_2 formed/mg DNA from E_1S) 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. *$P \leq 0.001$ vs. control value; **$P \leq 0.0005$ vs. control value. Quoted from Ref. [88].

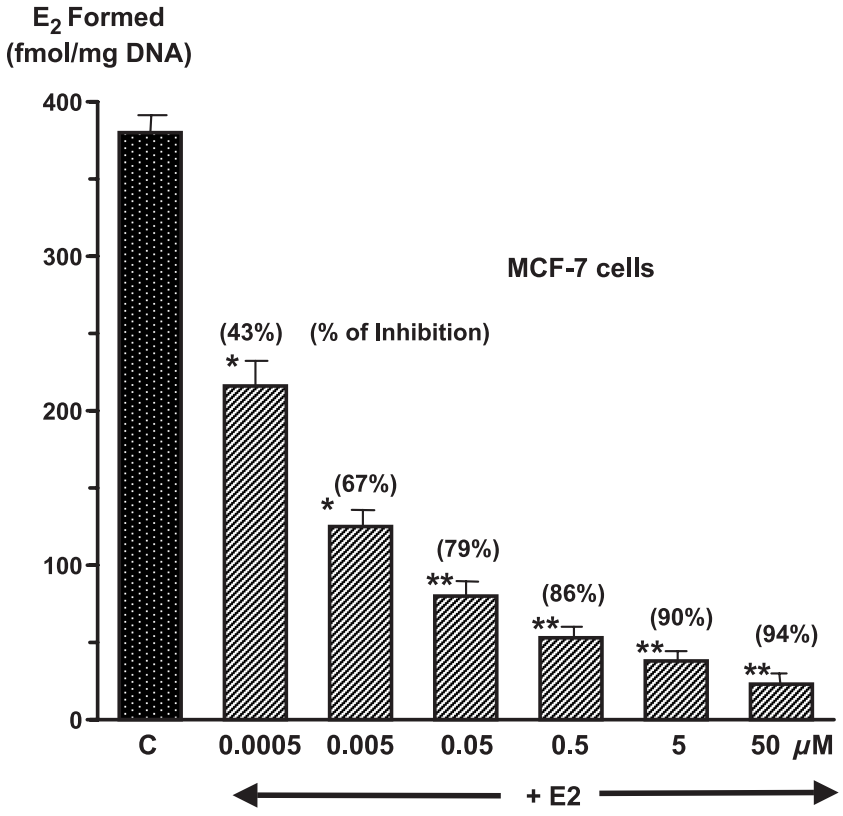


Fig. 6. Effect of estradiol (E_2) on the conversion of estrone sulfate (E_1S) to E_2 in the MCF-7 human breast cancer cells. The percentage of inhibition was obtained by calculating the ratio [(control − test)/control] × 100. The values are the mean ± S.E. of duplicate determinations of five independent experiments. *$P \leq 0.05$ vs. control value; **$P \leq 0.005$ vs. control value. Quoted from Ref. [104].

an IC_{50} 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 E_2 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 E_1S to E_2 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 IC_{50} 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

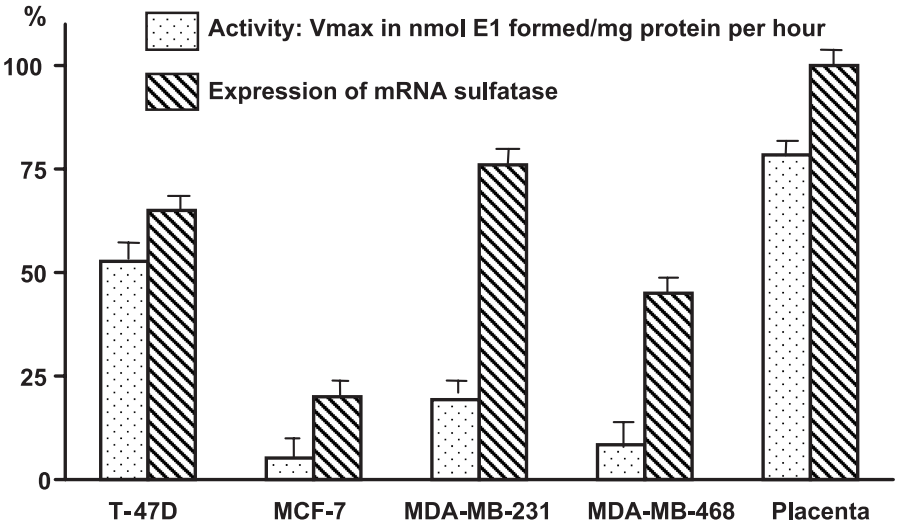


Fig. 7. Estrone sulfatase activity and relative expression of mRNA sulfatase in the hormone-dependent (MCF-7, T-47D) and hormone-independent (MDA-MB-231, MDA-MB-468) human breast cancer cell lines and in human term placenta. The estrone sulfatase activity was determined in the homogenates of the different cell lines and in the placenta under initial rate conditions. This activity was expressed as V_{max} (maximum velocity) using Lineweaver and Burk plot. Expression of mRNA sulfatase was analyzed by RT-PCR amplification and densitometry. The value of 100% was assigned to the mRNA expression of sulfatase in the placenta. The data represent the mean ± S.E. of two to five independent experiments. Quoted from Ref. [105].

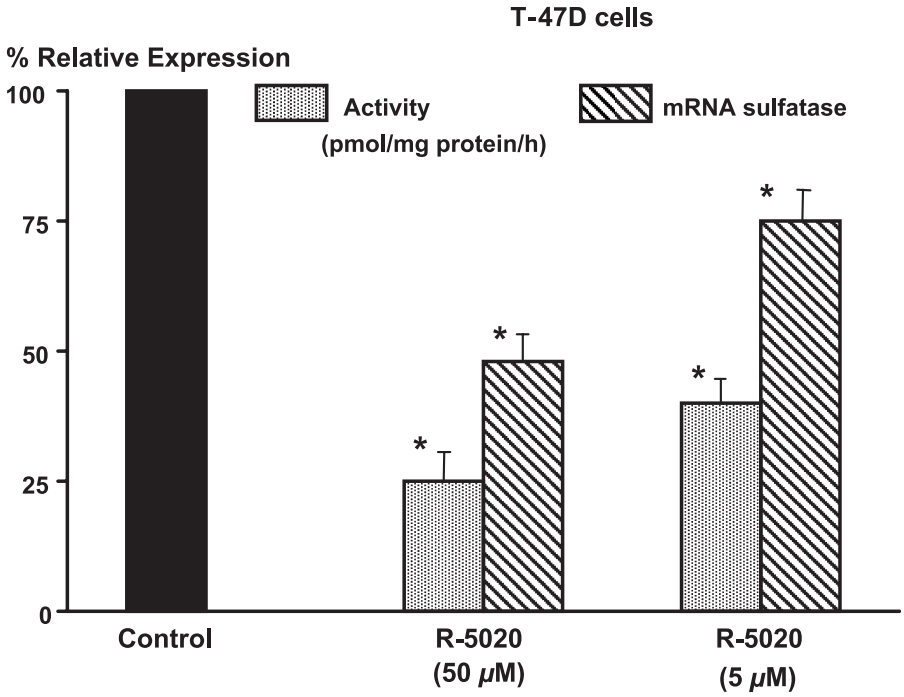


Fig. 8. Effects of the progestin R-5020 (promegestone) on the activity and the mRNA expression of estrone sulfatase in the hormone-dependent T-47D human breast cancer cell line. Relative expression of the mRNA (using RT-PCR) amplification) and the activity (in pmol/mg protein/h) of estrone sulfatase in T-47D cells non-treated (control) and treated with R-5020 at the concentrations of 5×10^{-5} or 5×10^{-6} mol/l. The control value is assigned 100%. The data represent the mean ± S.E. of three to four independent experiments. Quoted from Refs. [105,106].

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 E_2, sulfation occurs more rapidly with MCF-7 cells transformed with EST than with the control cells, thereby rendering E_2 physiologically inactive. EST/MCF-7 cells require a higher concentration of E_2 to stimulate growth than do control MCF-7 cells, as EST inactivates E_2 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 progesterone. 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 cells 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

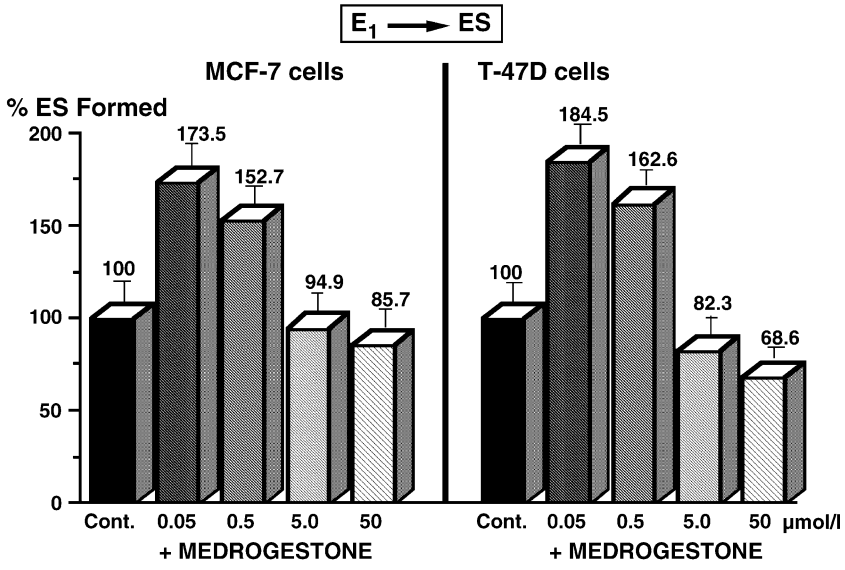


Fig. 9. Effects of Medrogestone (Prothil®) on the conversion of estrone (E_1) to estrogen sulfates in the hormone-dependent MCF-7 and T-47D human breast cancer cell lines. Preconfluent cells were incubated 24 h at 37 °C with 5×10^{-9} mol/l of [^{3}H]-E_1 alone (control; non-treated cells) or in the 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. *$P \leq 0.5$ vs. control value (non-treated cells); **$P \leq 0.01$ vs. control value (non-treated cells). Quoted from Ref. [126].

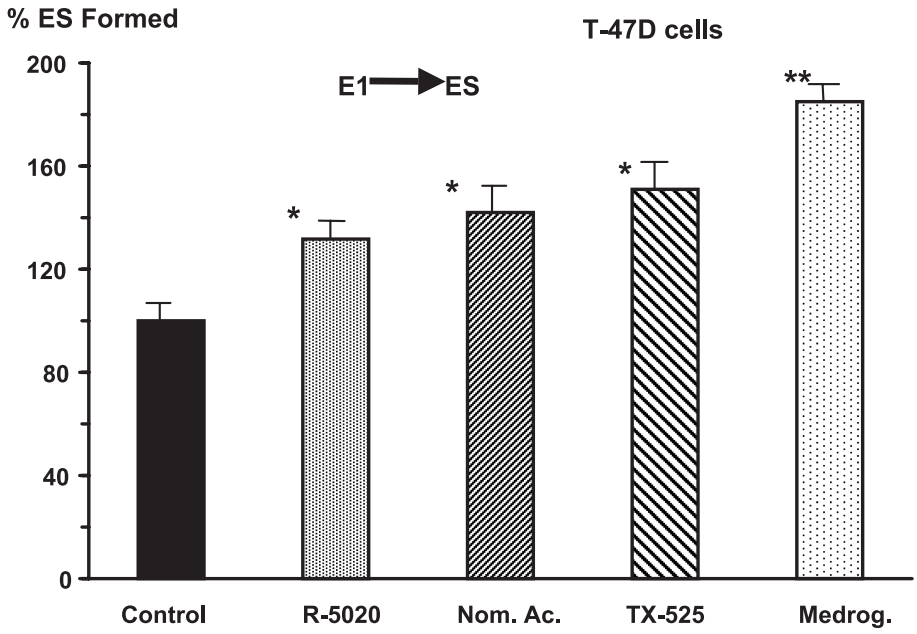


Fig. 10. Comparative effects of various progestins on the conversion of estrone (E_1) to estrogen sulfates (ES) in the hormone-dependent T-47D human breast cancer cell line. Preconfluent cells were incubated 24 h at 37 °C with 5×10^{-9} mol/l of [^{3}H]-E_1 alone (control; non-treated cells) or in the presence of progestins at the concentration of 5×10^{-8} mol/l. Results (pmol of ES formed in culture medium per mg DNA from E_1) are expressed in percent (%) of control value considered as 100%. The data represent the mean ± S.E. of duplicate determinations of three to six independent experiments. R-5020: promegestone; Nom.Ac.: nomegestrol acetate; TX-525: a 19-norprogestin of Theramex Laboratories; Medrog.: medrogestone. *$P \leq 0.05$ vs. control value; **$P \leq 0.01$ vs. control value.

cells. These compounds also provoke a dual effect on sulfotransferase activity: stimulatory at low doses (5×10^{-8} mol/l), whereas an inhibition of this activity is observed at higher doses (5×10^{-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

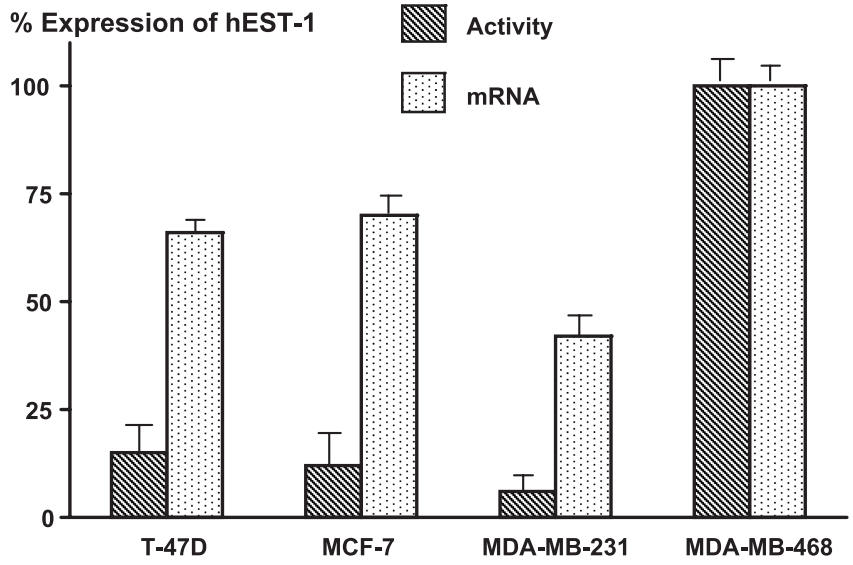


Fig. 11. Relative sulfotransferase activity and hEST type 1 mRNA expression in the hormone-dependent (MCF-7, T-47D) and hormone-independent (MDA-MB-231, MDA-MB-468) human breast cancer cell lines. Sulfotransferase activity was determined by incubation of 5×10^{-9} M of [^{3}H]-estrone ([^{3}H]-E_1) for 24 h at 37 °C with each cell line. The ES formed were only detectable in culture medium and were quantified by TLC method. Expression of mRNA EST was analyzed by RT-PCR amplification and densitometry Results were expressed in percent (%) and the value of 100% was assigned to the activity and the mRNA expression of EST in the MDA-MB-468 cells. The data represent the mean ± S.E. of three to five experiments. Quoted from Ref. [133].

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-

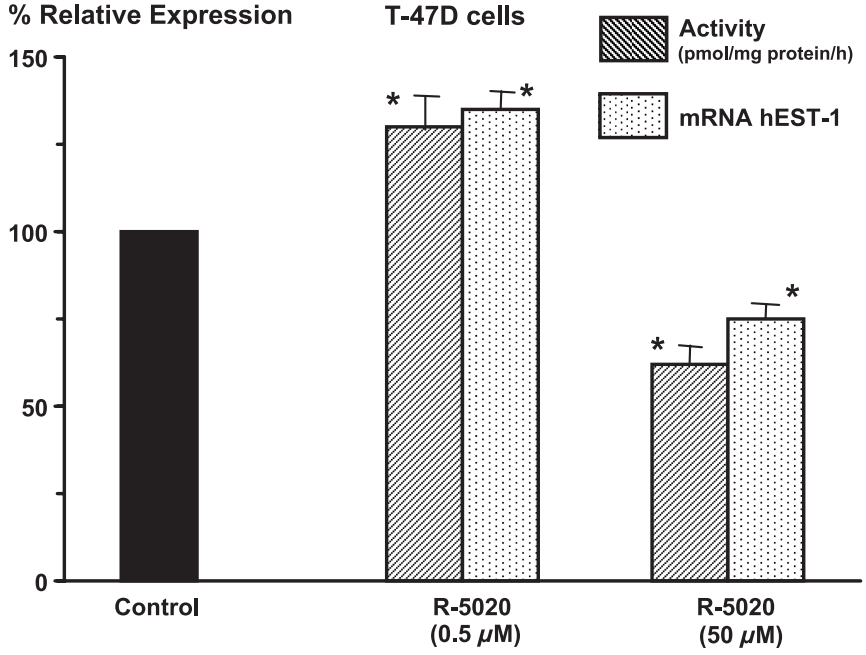
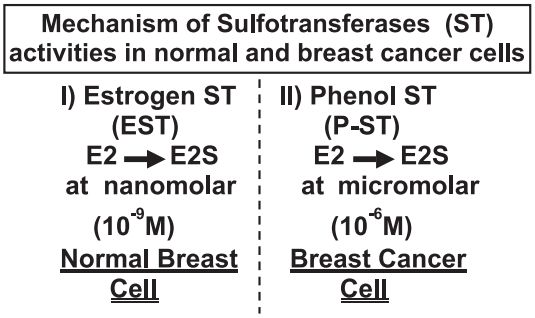
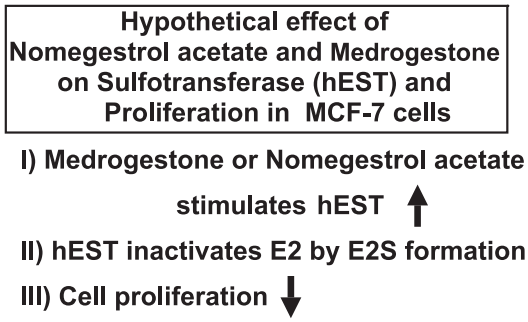


Fig. 12. Effects of the progestin R-5020 (promegestone) on the sulfotransferase activity and the mRNA expression of the estrogen sulfotransferase type 1 (hEST-1) in the hormone-dependent T-47D human breast cancer cell line. Relative expression of the mRNA (using RT-PCR amplification) and the activity (in pmol/mg protein/h) of hEST in T-47D cells non-treated (control) and treated with R-5020 at the concentration of 5×10^{-5} or 5×10^{-7}mol/l. The control value is assigned 100%. The data represent the mean ± S.E. of two to three experiments. Quoted from Ref. [133].



Scheme 1. Mechanism of sulfotransferase (ST) activity in normal and breast cancer cells. In normal breast cancer cells it is suggested that the action of hEST works at physiological (nanomolar) concentrations of estradiol to form estradiol sulfate which is biologically inactive. This enzyme is absent from breast cancer cells where the phenol-ST activity acts only at micromolar (non-physiological) concentrations.



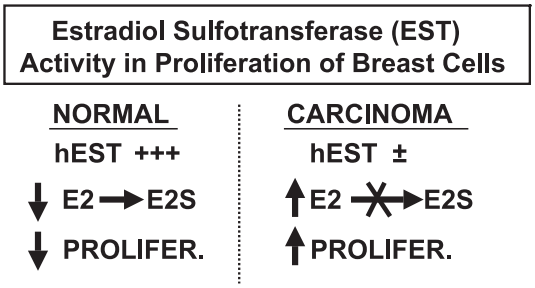
Scheme 3. Hypothetical effects of medrogestone on human sulfotransferase (hEST) and proliferation in T-47D and MCF-7 breast cancer cells. As medrogestone can stimulate hEST in the cancer cell, the effect of estradiol becomes inactive by the formation of estradiol sulfate and consequently cell proliferation is inhibited.

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 compound 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



Scheme 2. Effects of estradiol sulfotransferase (EST) activity on the proliferation of breast cancer cells. In the normal breast cancer cells, as a consequence of the hEST activity, the proliferation is inhibited, as estradiol sulfate (E_2S) is biologically inactive. In opposition to breast cancer cells, hEST activity is very low or inexistent as E_2S is not formed and E_2 can stimulate proliferation.

of estradiol, mainly present to form estradiol sulfate (E_2S) and consequently to block the proliferative effect of estradiol as E_2S, is biologically inactive. However, in the breast cancer cells the phenol sulfotransferase (P-ST), which is active at micromolar concentrations of E_2 (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 (E_2 to E_1) 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 (E_2 to E_1) 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 E_1 to E_2. 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 E_1) 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 (E_2 to E_1) 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 E_2 to E_1, 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 (E_1 to E_2) 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 (E_2 to E_1) 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 (E_2 to E_1) 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 IC_{50} values, which correspond to the 50% inhibition of the conversion of E_1 to E_2, 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

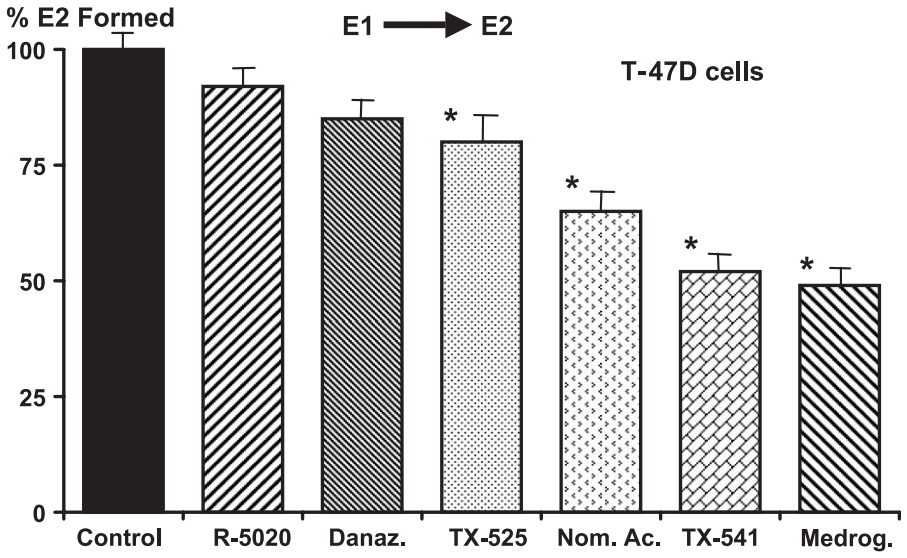


Fig. 13. Comparative effects of various progestins on the inhibition of the conversion of estrone (E_1) to estradiol (E_2) in the hormone-dependent T-47D human breast cancer cell line. Preconfluent cells were incubated 24 h at 37 °C with 5×10^{-9} mol/l of [^{3}H]-E_1 alone or in the presence of progestins at the concentration of 5×10^{-7} mol/l. Results (pmol of E_2 formed in cell compartment/mg DNA from E_1); 17β-HSD-1 activity) are expressed in percent (%) of control value considered as 100%. The data represent the mean ± S.E. of duplicate determinations of three to six independent experiments. R-5020: promegestone; Dana.: danazol; TX-525 and TX-541 are 19-norprogestins of Theramex Laboratories; Medrog.: medrogestone; Nom.Ac.: nomegestrol acetate. *$P \leq 0.05$ vs. control value.

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 5×10^{-7} M. The 3α-OH and 3β-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 5×10^{-6} M; The IC_{50} values in T-47D cells are respectively: 1.44, 2.03, 4.83, and 35.25 μM 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 (IC_{50} value: 890 μM) [80]. However, in our laboratory we found that ICI 164,384 at 5×10^{-6} M inhibits by 53% the conversion of E_1 to E_2 in T-47D cells [30].

Various potential irreversible or reversible inhibitors of 17β-HSD type 1 have been synthesized (e.g. bromoacetoxy or alkylamide derivatives of E_2 and of progesterone) [171–173]. Thus, for example, the compound 16α-(bromoalkylamide) derivative of E_2 inhibits the 17β-HSD type 1 in human placenta with an IC_{50} 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: aminoglutethimide, 4-hydroxy-androstenedione (Formestane; Lentaron®), Vorosole, Letrozole (Fermara®), 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

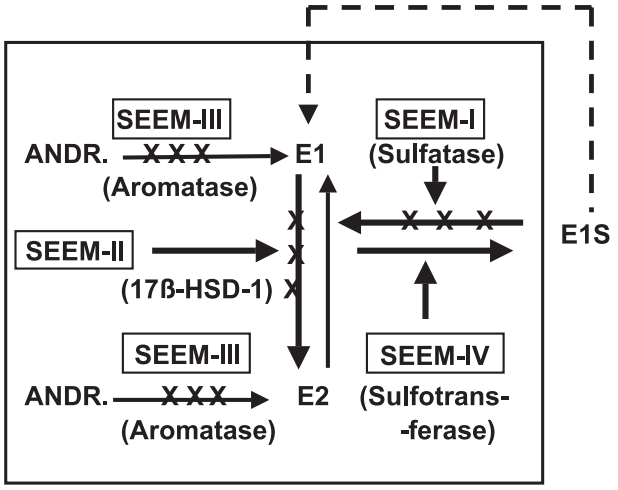


Fig. 14. The SEEM concept in human hormone-dependent breast cancer cells. The SEEM can control the enzymatic mechanisms involved in the formation and transformation of estrogens in breast cancer cells, where the sulfatase pathway is quantitatively higher than the aromatase. SEEM-I inhibits the estrone sulfatase; SEEM-II the 17β-HSD-1; SEEM-III the aromatase activities, and SEEM-IV stimulates the estrone sulfotransferase activity. It is suggested that E_1S is present in the tumor outside the cell and reaches the cell membrane where it is in contact with the intracellular estrone sulfatase. ANDR.: androgens; E_1: estrone; E_2: estradiol; E_1S: estrone sulfate.

shown that breast cancer patients treated with the anti-estrogen tamoxifen (Nolvadex) have a significantly reduced risk of recurrence and an increased overall survival. Recently, tests using a series of new anti-estrogens yielded very attractive clinical results. However, another way to block estradiol is by using anti-enzymes (anti-sulfatase, anti-aromatase, or anti-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 (E_2) 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 E_2 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.

Acknowledgements

The author would like to express deep thanks to Dr. G. Chetrite for help in developing the bibliography and to Ms. S. MacDonald for assistance in the preparation of this manuscript.

References

[1] A. Segaloff, Hormones and mammary carcinogenesis, in: W.L. McGuire (Ed.), Advances in Research and Treatment, Breast Cancer, vol. 2, Plenum, New York, 1978, pp. 1–22.
[2] M.A. Kirschner, The role of hormones in the development of human breast cancer, in: W.L. McGuire (Ed.), Advances in Research and Treatment, Current Topics, Breast Cancer, vol. 3, Plenum, New York, 1979, pp. 199–226.
[3] M.E. Lippman, R.B. Dickson, S. Bates, C. Knabbe, K. Huff, S. Swain, M. McManaway, D. Bronzert, A. Kasid, E.P. Gelmann, Autocrine and paracrine growth regulation of human breast cancer, Breast Cancer Res. Treat. 7 (1986) 59–70.
[4] B.E. Henderson, R. Ross, L. Bernstein, Estrogens as a cause of human cancer: the Richard and Hinda Rosenthal Foundation Award Lecture, Cancer Res. 48 (1988) 246–253.
[5] S. Raam, N. Robert, C.A. Pappas, H. Tamura, Defective estrogen receptors in human mammary cancers: their significance in defining hormone dependence, J. Natl. Cancer Inst. 80 (1988) 756–761.
[6] S.A.W. Fuqua, S.D. Fitzgerald, G.C. Chamness, A.K. Tandon, D.P. McDonnell, Z. Nawaz, B.W. O'Malley, W.L. McGuire, Variant human breast tumor estrogen receptor with constitutive transcriptional activity, Cancer Res. 51 (1991) 105–109.
[7] W.L. McGuire, G.C. Chamness, S.A.W. Fuqua, Abnormal estrogen receptor in clinical breast cancer, J. Steroid Biochem. Mol. Biol. 43 (1992) 243–247.
[8] S.A.W. Fuqua, G.L. Greene, W.L. McGuire, Inhibition of estrogen receptor action by a naturally occurring variant in human breast tumors, Cancer Res. 52 (1992) 483–486.
[9] C.G. Castles, S.A.W. Fuqua, D.M. Klotz, S.M. Hill, Expression of a constitutively active estrogen receptor variant in the estrogen receptor-negative BT-20 human breast cancer cell line, Cancer Res. 53 (1993) 5934–5939.
[10] D.P. Edwards, G.C. Chamness, W.L. McGuire, Estrogen and progesterone receptors in breast cancer, Biochim. Biophys. Acta 560 (1979) 457–486.

[11] S. Litherland, I.M. Jackson, Antiestrogens in the management of hormone-dependent cancer, Cancer Treat. Rev. 15 (1988) 183–194.
[12] J.M. Hall, M.K. Lee, B. Newman, J.E. Horrow, L.A. Anderson, B. Huey, M.C. King, Linkage of early-onset familial breast cancer to chromosome 17q21, Science 250 (1990) 1684–1689.
[13] C.S. Cropp, H.A. Nevanlinna, S. Pyrhönen, U.-H. Stenman, P. Salmikangas, H. Albertsen, R. White, R. Callahan, Evidence for involvement of BRCA1 in sporadic breast carcinomas, Cancer Res. 54 (1994) 2548–2551.
[14] B.A. Bove, R.L. Dunbrack Jr., A.K. Godwin, BRCA1, BRCA2, and hereditary breast cancer, in: J.R. Pasqualini (Ed.), Breast Cancer, Prognosis, Treatment, and Prevention, Marcel Dekker, New York, 2002, pp. 555–624.
[15] A.A.J. van Landeghem, J. Poortman, M. Nabuurs, J.H.H. Thijssen, Endogenous concentration and subcellular distribution of estrogens in normal and malignant human breast tissue, Cancer Res. 45 (1985) 2900–2906.
[16] A. Vermeulen, J.P. Deslypere, R. Paridaens, G. Leclercq, F. Roy, J.C. Heuson, Aromatase, 17β-hydroxysteroid dehydrogenase and intratissular sex hormone concentrations in cancerous and normal glandular breast tissue in postmenopausal women, Eur. J. Cancer Clin. Oncol. 22 (1986) 515–525.
[17] J.R. Pasqualini, G. Chetrite, C. Blacker, M.-C. Feinstein, L. Delalonde, M. Talbi, C. Maloche, Concentrations of estrone, estradiol, and estrone sulfate and evaluation of sulfatase and aromatase activities in pre- and postmenopausal breast cancer, J. Clin. Endocrinol. Metab. 81 (1996) 1460–1464.
[18] J.R. Pasqualini, J. Cortes-Prieto, G. Chetrite, M. Talbi, A. Ruiz, Concentrations of estrone, estradiol, and their sulfates and evaluation of sulfatase and aromatase activities in patients with breast fibroadenoma, Int. J. Cancer 70 (1997) 639–643.
[19] R. Clarke, F. Leonessa, J.N. Welch, T.C. Skaar, Cellular and molecular pharmacology of antiestrogen action and resistance, Pharmacol. Rev. 53 (2001) 1–47.
[20] Y.J. Abul-Hajj, R. Iverson, D.T. Kiang, Aromatization of androgens by human breast cancer, Steroids 33 (1979) 205–222.
[21] A. Lipton, S.J. Santner, R.J. Santen, H.A. Harvey, P.D. Feil, D. White-Hershey, M.J. Bartholomew, C.E. Antle, Aromatase activity in primary and metastatic human breast cancer, Cancer 59 (1987) 779–782.
[22] A.S. Bhatnagar, C. Batzl, A. Häusler, K. Schieweck, M. Lang, P.F. Trunet, Pharmacology of nonsteroidal aromatase inhibitors, in: J.R. Katzenellenbogen, B.S. Katzenellenbogen (Eds.), Hormone-Dependent Cancer, Marcel Dekker, New York, 1996, pp. 155–168.
[23] T.L. Dao, C. Hayes, P.R. Libby, Steroid sulfatase activities in human breast tumors, Proc. Soc. Exp. Biol. Med. 146 (1974) 381–384.
[24] F. Vignon, M. Terqui, B. Westley, D. Derocq, H. Rochefort, Effects of plasma estrogen sulfates in mammary cancer cells, Endocrinology 106 (1980) 1079–1086.
[25] J.R. Pasqualini, C. Gelly, F. Lecerf, Estrogen sulfates: biological and ultrastructural responses and metabolism in MCF-7 human breast cancer cells, Breast Cancer Res. Treat. 8 (1986) 233–240.
[26] J.R. MacIndoe, with the technical assistance of G. Woods, L. Jeffries, M. Hinkhouse, The hydrolysis of estrone sulfate and dehydroepiandrosterone sulfate by MCF-7 human breast cancer cells, Endocrinology 123 (1988) 1281–1287.
[27] J.R. Pasqualini, C. Gelly, B.-L. Nguyen, C. Vella, Importance of estrogen sulfates in breast cancer, J. Steroid Biochem. 34 (1989) 155–163.
[28] R.C. Bonney, M.J. Reed, K. Davidson, P.A. Beranek, V.H.T. James, The relationship between 17β-hydroxysteroid dehydrogenase activity and oestrogen concentrations in human breast tumours and in normal breast tissue, Clin. Endocrinol. 19 (1983) 727–739.
[29] J.M. McNeill, M.J. Reed, P.A. Beranek, R.C. Bonney, M.W. Ghilchik, D.J. Robinson, V.H.T. James, A comparison of the in vivo uptake and metabolism of ^{3}H-oestrone and ^{3}H-oestradiol by normal breast and breast tumour tissues in post-menopausal women, Int. J. Cancer 38 (1986) 193–196.
[30] B.-L. Nguyen, G. Chetrite, J.R. Pasqualini, Transformation of estrone and estradiol in hormone-dependent and hormone-independent human breast cancer cells. Effects of the antiestrogen ICI 164,384, danazol, and promegestone (R-5020), Breast Cancer Res. Treat. 34 (1995) 139–146.
[31] S.J. Santner, P.D. Feil, R.J. Santen, In situ estrogen production via the estrone sulfatase pathway in breast tumors: relative importance versus the aromatase pathway, J. Clin. Endocrinol. Metab. 59 (1984) 29–33.
[32] J.R. Pasqualini, Steroid sulphotransferase activity in human hormone-independent MDA-MB-468 mammary cancer cells, Eur. J. Cancer 28A (1992) 758–762.
[33] J.L. Falany, C.N. Falany, Expression of cytosolic sulfotransferases in normal mammary epithelial cells and breast cancer cell lines, Cancer Res. 56 (1996) 1551–1555.
[34] J.R. Pasqualini, F. Kincl, in: Hormones and the Fetus vol. 1, Pergamon, Oxford, 1985, pp. 173–334.
[35] R.A. Hawkins, R.E. Oakey, Estimation of oestrone sulphate, oestradiol-17β and oestrone in peripheral plasma: concentrations during the menstrual cycle and in man, J. Endocrinol. 60 (1974) 1–17.
[36] H. Honjo, J. Kitawaki, M. Itoh, J. Yasuda, K. Iwasaku, M. Urabe, K. Naitoh, T. Yamamoto, H. Okada, T. Ohkubo, T. Nambara, Serum and urinary estrone sulfate during the menstrual cycle, measured by a direct radio-immunoassay, and fate of exogenously injected estrone sulfate, Horm. Res. 27 (1987) 61–68.
[37] K.D. Roberts, J.G. Rochefort, G. Bleau, A. Chapdelaine, Plasma estrone sulfate levels in postmenopausal women, Steroids 35 (1980) 179–187.
[38] E. Samojlik, R.J. Santen, T.J. Worgul, Plasma estrone-sulfate assessment of reduced estrogen production during treatment of metastatic breast carcinoma, Steroids 39 (1982) 497–507.
[39] O.A. Towobolla, R.C. Crilly, R.E. Oakey, Oestrone sulphate in plasma from postmenopausal women and the effects of oestrogen and androgen therapy, Clin. Endocrinol. (Oxf.) 13 (1980) 461–471.
[40] O. Prost, M.O. Turrel, N. Dahan, C. Craveur, G.L. Adessi, Estrone and dehydroepiandrosterone sulfatase activities and plasma estrone sulfate levels in human breast carcinoma, Cancer Res. 44 (1984) 661–664.
[41] D.L. Loriaux, H.J. Ruder, M.B. Lipsett, The measurement of estrone sulfate in plasma, Steroids 18 (1971) 463–472.
[42] K. Wright, D.C. Collins, P.I. Musey, J.R.K. Preedy, A specific radioimmunoassay for estrone sulfate in plasma and uring without hydrolysis, J. Clin. Endocrinol. Metab. 47 (1978) 1092–1098.
[43] D. Drafta, A.E. Schindler, S.M. Milcu, E. Keller, E. Stroe, E. Horodniceanu, I. Balanescu, Plasma hormones in pre- and postmenopausal breast cancer, J. Steroid Biochem. 13 (1980) 793–802.
[44] R.C. Stein, M. Dowsett, A. Hedley, J.-C. Gazet, H.T. Ford, R.C. Coombes, The clinical and endocrine effects of 4-hydroxyandrostenedione alone and in combination with goserelin in premenopausal women ith advanced breast cancer, Br. J. Cancer 62 (1990) 679–683.
[45] R.W. Blamey, W. Jonat, M. Kaufmann, A.R. Bianco, M. Namer, Goserelin depot in the treatment of premenopausal advanced breast cancer, Eur. J. Cancer 28A (1992) 810–814.
[46] Z.B. Neskovic-Konstantinovic, L.B. Vuletic, L.I. Nikolic-Stanojevic, S.V. Susnjar, S.B. Jelic, M.V. Brankovic-Magic, S.S. Radulovic, Therapeutic and endocrine effects of Decapeptyl®, synthetic LH-RH agonistic analogue in premenopausal women with metastatic breast cancer—a pilot phase II study, Oncology 51 (1994) 95–101.
[47] M. Massobrio, M. Migliardi, P. Cassoni, C. Menzaghi, A. Revelli, G. Cenderelli, Steroid gradients across the cancerous breast: an index of altered steroid metabolism in breast cancer? J. Steroid Biochem. Molec. Mol. 51 (1994) 175–181.
[48] H.J. Ruder, L. Loriaux, M.B. Lipsett, Estrone sulfate: production rate and metabolism in man, J. Clin. Invest. 51 (1972) 1020–1033.
[49] C.T. Noel, M.J. Reed, H.S. Jacobs, V.H.T. James, The plasma con-

centration of oestrone sulphate in postmenopausal women: lack of diurnal variation, effect of ovariectomy, age and weight, J. Steroid Biochem. 14 (1981) 1101–1105.
[50] M.J. Reed, R.W. Cheng, C.T. Noel, H.A.F. Dudley, V.H.T. James, Plasma levels of estrone, estrone sulfate, and estradiol and the percentage of unbound estradiol in postmenopausal women with and without breast disease, Cancer Res. 43 (1983) 3940–3943.
[51] R.A. Hawkins, M.L. Thomson, E. Killen, Oestrone sulphate, adipose tissue, and breast cancer, Breast Cancer Res. Treat. 6 (1985) 75–87.
[52] P.E. Lonning, D.C. Johannessen, T. Thorsen, Alterations in the production rate and the metabolism of oestrone and oestrone sulphate in breast cancer patients treated with aminoglutethimide, Br. J. Cancer 60 (1989) 107–111.
[53] C. Recchione, E. Venturelli, A. Manzari, A. Cavalleeri, A. Martinetti, G. Secreto, Testosterone, dihydrotestosterone and oestradiol levels in postmenopausal breast cancer tissues, J. Steroid Biochem. Mol. Biol. 52 (1995) 541–546.
[54] A.A.J. van Landeghem, J. Poortman, M. Nabuurs, J.H.H. Thijssen, Endogenous concentration and subcellular distribution of estrogens in normal and malignant human breast tissue, Cancer Res. 45 (1985) 2900–2906.
[55] J.R. Pasqualini, G. Chetrite, B.-L. Nguyen, C. Blacker, M.-C. Feinstein, C. Maloche, M. Talbi, L. Delalonde, Control of estrone sulfatase activity and its expression in human breast cancer, in: M. Motta, M. Serio (Eds.), Sex Hormones and Antihormones in Endocrine-Dependent Pathology: Basic and Clinical Aspects, Excerpta Medica Int. Congr. Series, vol. 1064, 1994, pp. 257–265.
[56] J.H.H. Thijssen, M.A. Blankenstein, Endogenous oestrogens and androgens in normal and malignant endometrial and mammary tissues, Eur. J. Cancer Clin. Oncol. 25 (1989) 1953–1959.
[57] M. Assicot, G. Contesso, C. Bohuon, Catechol-o-methyltransferase in human breast cancer, Eur. J. Cancer 13 (1977) 961–966.
[58] Y.J. Abul-Hajj, J.H.H. Thijssen, M.A. Blankenstein, Metabolism of estradiol by human breast cancer, Eur. J. Cancer Clin. Oncol. 24 (1988) 1171–1178.
[59] B.T. Zhu, A.H. Conney, Is 2-methoxyestradiol an endogenous estrogen metabolite that inhibits mammary carcinogenesis, Cancer Res. 58 (1998) 2269–2277.
[60] M.-L. Lottering, M. Haag, J.C. Seegers, Effects of 17β-estradiol metabolites on cell cycle events in MCF-7 and HeLa cells, Cancer Res. 52 (1992) 5926–5932.
[61] C. Lippert, H. Seeger, A.O. Mueck, The effect of endogenous estradiol metabolites on the proliferation of human breast cancer cells, Life Sci. 72 (2003) 877–883.
[62] G.R. Merriam, N.J. MacLusky, M.K. Picard, F. Naftolin, Comparative properties of the catechol estrogens: I. Methylation by catechol-*O*-methyltransferase and binding to cytosol estrogen receptors, Steroids 36 (1980) 1–11.
[63] N.J. Lakhani, M.A. Sarkar, J. Venitz, W.D. Figg, 2-Methoxyestradiol, a promising anticancer agent, Pharmacotherapy 23 (2003) 165–172.
[64] N. Schütze, G. Vollmer, I. Tiemann, M. Geiger, R. Knuppen, Cate cholestrogens are MCF-7 cell estrogen receptor agonists, J. Steroid Biochem. Mol. Biol. 46 (1993) 781–789.
[65] A.O. Mueck, H. Seeger, T.H. Lippert, Estradiol metabolism and malignant disease, Maturitas 43 (2002) 1–10.
[66] L.A. Castagnetta, O.M. Granata, F.P. Arcuri, L.M. Polito, F. Rosati, G.P. Cartini, Gas chromatography/mass spectrometry of catechol estrogens, Steroids 57 (1992) 437–443.
[67] J.G. Liehr, M.J. Ricci, 4-Hydroxylation of estrogens as marker of human mammary tumors, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 3294–3296.
[68] J. Fishman, C. Martucci, Biological properties of 16α-hydroxyestrone: implications in estrogen physiology and pathophysiology, J. Clin. Endocrinol. Metab. 51 (1980) 611–615.
[69] A. Suto, H.L. Bradlow, G.Y. Wong, M.P. Osborne, N.T. Telang, Persistent estrogen responsiveness of ras oncogene-transformed mouse mammary epithelial cells, Steroids 57 (1992) 262–268.
[70] N.T. Telang, R. Narayanan, H.L. Bradlow, M.P. Osborne, Coordinated expression of intermediate biomarkers for tumorigenic transformation in ras-transfected mouse mammary epithelial cells, Breast Cancer Res. Treat. 18 (1991) 155–163.
[71] J.S. Lewis, T.J. Thomas, C.M. Klinge, M.A. Gallo, T. Thomas, Regulation of cell cycle and cyclins by 16alpha-hydroxyestrone in MCF-7 breast cancer cells, J. Molec. Endocrinol. 27 (2001) 293–307.
[72] A.M.H. Brodie, L.Y. Wing, M. Dowsett, R.C. Coombes, Aromatase inhibitors and treatment of breast cancer, J. Steroid Biochem. 24 (1986) 91–97.
[73] P.C. de Jong, J. van de Ven, H.W.R. Nortier, I. Maitimu-Smeele, T.H. Donker, J.H.H. Thijssen, P.H.T.J. Slee, R.A. Blankenstein, Inhibition of breast cancer tissue aromatase activity and estrogen concentrations by the third-generation aromatase inhibitor Vorozole, Cancer Res. 57 (1997) 2109–2111.
[74] H. Fujikawa, F. Okura, Y. Kuwano, A. Sekizawa, H. Chiba, K. Saito, H. Saito, T. Yanaihara, Steroid sulfatase activity in osteoblast cells, Biochem. Biophys. Res. Commun. 231 (1997) 42–47.
[75] G. Parenti, G. Meroni, A. Ballabio, The sulfatase gene family, Curr. Opin. Genet. Dev. 7 (1997) 386–391.
[76] P.J. Hughes, L.E. Twist, J. Durham, M.A. Choudhry, M. Drayson, R. Chandraratna, R.H. Michell, C.J. Kirk, G. Brown, Up-regulation of steroid sulphatase activity in HL60 promyelocytic cells by retinoids and 1alpha,25-dihydroxyvitamin D_3, Biochem. J. 355 (2001) 361–371.
[77] J.R. Pasqualini, C. Gelly, Effect of tamoxifen and tamoxifen derivatives on the conversion of estrone-sulfate to estradiol in the MCF-7 and R-27 mammary cancer cell lines, Cancer Lett. 40 (1988) 115–121.
[78] J.R. Pasqualini, N. Giambiagi, C. Gelly, G. Chetrite, Antiestrogen action in mammary cancer and in fetal cells, J. Steroid Biochem. Mol. Biol. 37 (1990) 343–348.
[79] J.R. Pasqualini, B.-L. Nguyen, Estrone sulfatase activity and effect of antiestrogens on transformation of estrone sulfate in hormone-dependent vs. independent human breast cancer cell lines, Breast Cancer Res. Treat. 18 (1991) 93–98.
[80] S.J. Santner, R.J. Santen, Inhibition of estrone sulfatase and 17β-hydroxysteroid dehydrogenase by antiestrogens, J. Steroid Biochem. Mol. Biol. 45 (1993) 383–390.
[81] J.R. Pasqualini, B. Schatz, C. Varin, B.-L. Nguyen, Recent data on estrogen sulfatases and sulfotransferases activities in human breast cancer, J. Steroid Biochem. Mol. Biol. 41 (1992) 323–329.
[82] J.R. Pasqualini, C. Varin, B.-L. Nguyen, Effect of the progestagen R-5020 (Promegestone) and of progesterone on the uptake and on the transformation of estrone sulfate in the MCF-7 and T-47D human mammary cancer cells: correlation with progesterone receptor levels, Cancer Lett. 66 (1992) 55–60.
[83] G. Chetrite, J. Paris, J. Botella, J.R. Pasqualini, Effect of nomegestrol acetate on estrone-sulfatase and 17β-hydroxysteroid dehydrogenase activities in human breast cancer cells, J. Steroid Biochem. Mol. Biol. 58 (1996) 525–531.
[84] G. Chetrite, C. Ebert, F. Wright, J.-C. Philippe, J.R. Pasqualini, Control of sulfatase and sulfotransferase activities by medrogestone in the hormone-dependent MCF-7 and T-47D human breast cancer cell lines, J. Steroid Biochem. Mol. Biol. 70 (1999) 39–45.
[85] J.R. Pasqualini, P. Caubel, A.J. Friedman, J.-C. Philippe, G. Chetrite, Norelgestromin as selective estrogen enzyme modulator in human breast cancer cell lines. Effect on sulfatase activity in comparison to medroxyprogesterone acetate, J. Steroid Biochem. Mol. Biol. 84 (2003) 193–198.
[86] J.R. Pasqualini, Differential effects of progestins on breast tissue enzymes, Maturitas 46 (S1) (2003) S7–S16.
[87] J.R. Pasqualini, G. Chetrite, The selective estrogen enzyme modulators (SEEM) in breast cancer, in: J.R. Pasqualini (Ed.), Breast Cancer: Prognosis, Treatment and Prevention, Marcel Dekker, New York, 2002, pp. 187–250.
[88] G. Chetrite, H.J. Kloosterboer, J.R. Pasqualini, Effect of tibolone (Org OD14) and its metabolites on estrone sulphatase activity in

MCF-7 and T-47D mammary cancer cells, Anticancer Res. 17 (1997) 135–140.
[89] J.R. Pasqualini, H.J. Kloosterboer, G.S. Chetrite, Effect of tibolone and its two hydroxy metabolites on estrone sulfatase activity in human breast cancer tissue, Breast Cancer Res. Treat. 76 (Suppl. 1) (2002) S99(Abst. 371).
[90] K.W. Selcer, P.-K. Li, Estrogenicity, antiestrogenecity and estrone sulfatase inhibition of estrone-3-amine and estrone-3-thiol, J. Steroid Biochem. Mol. Biol. 52 (1995) 281–286.
[91] N.M. Howarth, A. Purohit, M.J. Reed, B.V.L. Potter, Estrone sulfamates: potent inhibitors of estrone sulfatase with therapeutic potential, J. Med. Chem. 37 (1994) 219–221.
[92] A. Purohit, G.J. Williams, C.J. Roberts, B.V.L. Potter, M.J. Reed, In vivo inhibition of oestrone sulphatase and dehydroepiandrosterone sulphatase by oestrone-3-*O*-sulphamate, Int. J. Cancer 63 (1995) 106–111.
[93] W. Elger, S. Schwarz, A. Hedden, G. Reddersen, B. Schneider, Sulfamates of various estrogens are prodrugs with increased systemic and reduced hepatic estrogenicity at oral application, J. Steroid Biochem. Mol. Biol. 55 (1995) 395–403.
[94] S.D. Valigora, P.-K. Li, G. Dunphy, M. Turner, D.L. Ely, Steroid sulfatase inhibitor alters blood pressure and steroid profiles in hypertensive rats, J. Steroid Biochem. Mol. Biol. 73 (2000) 113–122.
[95] C.J. Anderson, L.J.H. Lucas, T.S. Widlanski, Molecular recognition in biological systems: phosphate esters vs. sulfate esters and the mechanism of action of steroid sulfatase, J. Am. Chem. Soc. 117 (1995) 3889–3890.
[96] R.P. Boivin, V. Luu-The, R. Lachance, F. Labrie, D. Poirier, Structure–activity relationships of 17alpha-derivates of estradiol as inhibitors of steroid-sulfatase, J. Med. Chem. 43 (2000) 4465–4478.
[97] D. Poirier, R.P. Boivin, 17α-alkyl- or 17α-substituted benzyl-17β-estradiols: a new family of estrone-sulfatase inhibitors, Bioorg. Med. Chem. Lett. 8 (1998) 1891–1896.
[98] L.C. Ciobanu, R.P. Boivin, V. Luu-The, F. Labrie, D. Poirier, Potent inhibition of steroid sulfatase activity by 3-*O*-sulfamate 17α-benzyl (or 4′ -tert-butylbenzyl)estra-1,3,5(10)-trienes: combinaison of two substituents at positions C3 and C17α of estradiol, J. Med. Chem. 42 (1999) 2280–2286.
[99] A. Purohit, K.A. Vernon, A.E.W. Hummelinck, L.W.L. Woo, H.A.M. Hejaz, B.V.L. Potter, M.J. Reed, The development of a-ring modified analogues of oestrone-3-*O*-sulphamate as potent steroid sulphatase inhibitors with reduce oestrogenecity, J. Steroid Biochem. Mol. Biol. 64 (1998) 269–276.
[100] A. Purohit, L.W.L. Woo, D. Barrow, H.A.M. Hejaz, R.I. Nicholson, B.V.L. Potter, M.J. Reed, Non-steroidal and steroidal sulfamates: new drugs for cancer therapy, Mol. Cell. Endocrinol. 171 (2001) 129–135.
[101] B. Malini, A. Purohit, D. Ganeshapillai, L.W.L. Woo, B.V.L. Potter, M.J. Reed, Inhibition of steroid sulphatase activity by tricyclic coumarin sulphamates, J. Steroid Biochem. Mol. Biol. 75 (2000) 253–258.
[102] L.W.L. Woo, M. Lightowler, A. Purohit, M.J. Reed, B.V.L. Potter, Heteroatom-substituted analogues of the active-site directed inhibitor estra-1,3,5(10)-trien-17-one-3-sulphamate inhibit estrone sulphatase by a different mechanism, J. Steroid Biochem. Mol. Biol. 57 (1996) 79–88.
[103] L.W.L. Woo, A. Purohit, B. Malini, M.J. Reed, B.V.L. Potter, Potent active site-directed inhibition of steroid sulphatase by tricyclic coumarin-based sulphamates, Chem. Biol. 7 (2000) 773–791.
[104] J.R. Pasqualini, G. Chetrite, Paradoxical effect of estradiol: it can block its own bioformation in human breast cancer cells, J. Steroid Biochem. Mol. Biol. 78 (2001) 21–24.
[105] J.R. Pasqualini, C. Maloche, M. Maroni, G. Chetrite, Effect of the progestagen promegestone (R-5020) on mRNA of the oestrone sulphatase in the MCF-7 human mammary cancer cells, Anticancer Res. 14 (1994) 1589–1593.
[106] J.R. Pasqualini, G. Chetrite, E. Le Nestour, Control and expression of oestrone sulphatase activities in human breast cancer, J. Endocrinol. 150 (1996) S99–S105.
[107] G. Chetrite, C. Varin, L. Delalonde, J.R. Pasqualini, Effect of promegestone, tamoxifen, 4-hydroxytamoxifen and ICI 164,384 on the oestrone sulphatase activity of human breast cancer cells, Anticancer Res. 13 (1993) 931–934.
[108] S.P. Newman, A. Purohit, M.W. Ghilchick, B.V.L. Potter, M.J. Reed, Regulation of steroid sulphatase expression and activity in breast cancer, J. Steroid Biochem. Mol. Biol. 75 (2000) 259–264.
[109] T. Utsumi, N. Yoshimura, S. Takeuchi, M. Maruta, K. Maeda, N. Harada, Elevated steroid sulfatase expression in breast cancers, J. Steroid Biochem. Mol. Biol. 73 (2000) 141–145.
[110] Y. Miyoshi, A. Ando, E. Shiba, T. Taguchi, Y. Tamaki, S. Noguchi, Involvement of up-regulation of 17beta-hydroxysteroid dehydrogenase type 1 in maintenance of intratumoral high estradiol levels in postmenopausal breast cancers, Int. J. Cancer 94 (2001) 685–689.
[111] I.A. Aksoy, R. Wood, R. Weinshilboum, Human liver estrogen sulfotransferase: identification by cDNA cloning and expression, Biochem. Biophys. Res. Commun. 200 (1994) 1621–1629.
[112] F. Faucher, L. Lacoste, I. Dufort, V. Luu-The, High metabolization of catecholestrogens by type 1 estrogen sulfotransferase (h EST 1), J. Steroid Biochem. Mol. Biol. 77 (2001) 83–86.
[113] M.J. Wild, P.S. Rudland, D.J. Back, Metabolism of the oral contraceptive steroids ethynylestradiol and norgestimate by normal (Huma 7) and malignant (MCF-7 and ZR 75-1) human breast cells in culture, J. Steroid Biochem. Mol. Biol. 39 (1991) 535–543.
[114] E. Anderson, A. Howell, Oestrogen sulphotransferases in malignant and normal human breast tissue, Endocr.-Relat. Cancer 2 (1995) 227–233.
[115] J. Bartek, J. Bartkova, N. Kyprianou, E.-L. Lalani, Z. Staskova, M. Shearer, S. Chang, J. Taylor-Papadimitriou, Efficient immortalization of luminal epithelial cells from human mammary gland by introduction of simian virus 40 large tumour antigen with a recombinant retrovirus, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 3520–3524.
[116] E. Leygue, R. Gol-Winkler, A. Gompel, C. Louis-Sylvestre, L. Soquet, S. Staub, F. Kuttenn, P. Mauvais-Jarvis, Estradiol stimulates c-myc proto-oncogene expression in normal human breast epithelial cells in culture, J. Steroid Biochem. Mol. Biol. 52 (1995) 299–305.
[117] C. Malet, A. Gompel, H. Yaneva, H. Cren, N. Fidji, I. Mowszowicz, F. Kuttenn, P. Mauvais-Jarvis, Estradiol and progesterone receptors in culture normal breast epithelial cells and fibroblasts: immunocytochemical studies, J. Clin. Endocrinol. Metab. 73 (1991) 8–17.
[118] Y. Qian, C. Deng, W.-C. Song, Expression of estrogen sulfotransferase in MCF-7 cells by cDNA transfection suppresses the estrogen response: potential role of the enzyme in regulating estrogen-dependent growth of breast epithelial cells, J. Pharmacol. Exp. Ther. 286 (1998) 555–560.
[119] J.L. Falany, L. Lawing, C.N. Falany, Identification and characterization of cytosolic sulfotransferase activities in MCF-7 human breast carcinoma cells, J. Steroid Biochem. Mol. Biol. 46 (1993) 481–487.
[120] L. Tseng, J. Mazella, L.Y. Lee, M.L. Stone, Estrogen sulfatase and estrogen sulfotransferase in human primary mammary carcinoma, J. Steroid Biochem. 19 (1983) 1413–1417.
[121] J.B. Adams, N.S. Phillips, Properties of estrogen and hydroxysteroid sulphotransferases in human mammary cancer, J. Steroid Biochem. 36 (1990) 695–701.
[122] S. Sharp, J.M. Anderson, M.W.H. Coughtrie, Immunohistochemical localisation of hydroxysteroid sulphotransferase in human breast carcinoma tissue: a preliminary study, Eur. J. Cancer 30A (1994) 1654–1658.
[123] C.N. Falany, J. Wheeler, T.S. Oh, J.L. Falany, Steroid sulfation by expressed human cytosolic sulfotransferases, J. Steroid Biochem. Mol. Biol. 48 (1994) 369–375.
[124] J.L. Falany, C.N. Falany, Regulation of estrogen activity by sulfation in human MCF-7 human breast cancer cells, Oncol. Res. 9 (1997) 589–596.
[125] G.S. Chetrite, C. Ebert, F. Wright, J.-C. Philippe, J.R. Pasqualini,

Effect of medrogestone on 17β-hydroxysteroid dehydrogenase activity in the hormone-dependent MCF-7 and T-47D human breast cancer cell lines, J. Steroid Biochem. Mol. Biol. 68 (1999) 51–56.
[126] G.S. Chetrite, C. Ebert, F. Wright, J.-C. Philippe, J.R. Pasqualini, Control of sulfatase and sulfotransferase activities by medrogestone in the hormone-dependent MCF-7 and T-47D human breast cancer cells lines, J. Steroid Biochem. Mol. Biol. 70 (1999) 39–45.
[127] P.M. Kicovic, J. Cortes-Prieto, M. Luisi, F. Franchi, Placebo-controlled cross-over study of the effects of Org OD 14 in menopausal women, Reproduccion 6 (1982) 81–91.
[128] N.H. Bjarnason, K. Bjarnason, J. Haarbo, C. Christiansen, Tibolone: prevention of bone loss in late postmenopausal women, J. Clin. Endocrinol. Metab. 81 (1996) 2422–3219.
[129] G.S. Chetrite, H.J. Kloosterboer, J.-C. Philippe, J.R. Pasqualini, Effect of Org OD14 (Livial®) and its metabolites on human estrogen sulphotransferase activity in the hormone-dependent MCF-7 and T-47D, and the hormone-independent MDA-MB-231, breast cancer cells lines, Anticancer Res. 19 (1999) 269–276.
[130] C.N. Falany, V. Krasnykh, J.L. Falany, Bacterial expression and characterization of a cDNA for human liver estrogen sulfotransferase, J. Steroid Biochem. Mol. Biol. 52 (1995) 529–539.
[131] F. Bernier, G. Leblanc, F. Labrie, V. Luu-The, Structure of human estrogen and aryl sulfotransferase gene, J. Biol. Chem. 269 (1994) 28200–28205.
[132] V. Luu-The, F. Bernier, I. Dufort, Steroid sulfotransferases, J. Endocrinol. 150 (1996) S87–S97.
[133] G. Chetrite, E. Le Nestour, J.R. Pasqualini, Human estrogen sulfotransferase (hEST1) activities and its mRNA in various breast cancer cell lines. Effect of the progestin, promegestone (R-5020), J. Steroid Biochem. Mol. Biol. 66 (1998) 295–302.
[134] T.A. Longacre, S.A. Bartow, A correlative morphologic study of human breast and endometrium in the menstrual cycle, Am. J. Surg. Pathol. 10 (1986) 382–393.
[135] Y.J. Topper, C.S. Freeman, Multiple hormone interactions in the developmental biology of the mammary gland, Physiol. Rev. 60 (1980) 1049–1106.
[136] J. Russo, I.H. Russo, Mechanisms involved in carcinogenesis of the breast, in: J.R. Pasqualini (Ed.), Breast Cancer, Prognosis, Treatment, and Prevention, Marcel Dekker, New York, 2002, pp. 555–624.
[137] I.J. Laidlaw, R.B. Clarke, A. Howell, A.W.M.C. Owen, C.S. Potten, E. Anderson, The proliferation of normal human breast tissue implanted into athymic nude mice is stimulated by estrogen but not progesterone, Endocrinology 136 (1995) 164–171.
[138] A. Gompel, C. Malet, P. Spritzer, J.-P. Lalardrie, F. Kuttenn, P. Mauvais-Jarvis, Progestin effect on cell proliferation and 17β-hydroxysteroid dehydrogenase activity in normal human breast cells in culture, J. Clin. Endocrinol. Metab. 63 (1986) 1174–1180.
[139] C. Malet, P. Spritzer, D. Guillaumin, F. Kuttenn, Progesterone effect on cell growth, ultrastructural aspect and estradiol receptors of normal human breast epithelial (HBE) cells in culture, J. Steroid Biochem. Mol. Biol. 73 (2000) 171–181.
[140] M.J. McManus, C.W. Welsch, The effect of estrogen, progesterone, thyroxine, and human placental lactogen on DNA synthesis of human breast ductal epithelium maintained in athymic nude mice, Cancer 54 (1984) 1920–1927.
[141] S.M. Longman, G.C. Buehring, Oral contraceptives and breast cancer. In vitro effect of contraceptive steroids on human mammary cell growth, Cancer 59 (1987) 281–287.
[142] F. Vignon, S. Bardon, D. Chalbos, H. Rochefort, Antiestrogenic effect of R5020, a synthetic progestin in human breast cancer cells in culture, J. Clin. Endocrinol. Metab. 56 (1983) 1124–1130.
[143] K.B. Horwitz, G.R. Freidenberg, Growth inhibition and increase of insulin receptors in antiestrogen-resistant T-47Dco human breast cancer cells by progestins: implication for endocrine therapies, Cancer Res. 45 (1985) 167–173.
[144] E.A. Musgrove, C.S. Lee, R.L. Sutherland, Progestins both stimulate and inhibit breast cancer cell cycle progression while increasing expression of transforming growth factor alpha, epidermal growth factor receptor, c-fos and c-myc genes, Mol. Cell. Biol. 11 (1991) 5032–5043.
[145] J. Botella, E. Duranti, I. Duc, A.M. Cognet, R. Delansorne, J. Paris, Inhibition by nomegestrol acetate and other synthetic progestins on proliferation and progesterone receptor content of T47-D human breast cancer cells, J. Steroid Biochem. Mol. Biol. 50 (1994) 41–47.
[146] M.-H. Jeng, C.J. Parker, V.C. Jordan, Estrogenic potential in oral contraceptives to stimulate human breast cancer cell proliferation, Cancer Res. 52 (1992) 6539–6546.
[147] W.H. Catherino, V.C. Jordan, Nomegestrol acetate, a clinically useful 19-norprogesterone derivative which lacks estrogenic activity, J. Steroid Biochem. Mol. Biol. 55 (1995) 239–246.
[148] E. Kalkhoven, L. Kwakkenbos-Isbrücker, S.W. De Latt, P.T. van der Saag, B. van den Burg, Synthetic progestins induce proliferaton of breast tumor cell lines via the progesterone or estrogen receptor, Mol. Cell. Endocrinol. 102 (1994) 45–52.
[149] R.W. Schatz, A.M. Soto, C. Sonnenschein, Effects of interaction between estradiol-17β and progesterone on the proliferation of cloned breast tumor cells (MCF-7 and T-47D), J. Cell. Physiol. 124 (1985) 386–390.
[150] K. Pollow, E. Boquoi, J. Baumann, M. Schmidt-Gollwitzer, B. Pollow, Comparison of the in vitro conversion of estradiol-17β to estrone of normal and neoplastic human breast tissue, Mol. Cell. Endocrinol. 6 (1977) 333–348.
[151] M. Miettinen, M. Mustonen, M. Poutanen, V. Isomaa, M. Wickman, G. Söderqvist, R. Vihko, P. Vihko, 17β-Hydroxysteroid dehydrogenases in normal human mammary epithelial cells and breast tissue, Breast Cancer Res. Treat. 57 (1999) 175–182.
[152] J.F. Prudhomme, C. Malet, A. Gompel, J.P. Lalardie, C. Ochoa, A. Boue, P. Mauvais-Jarvis, F. Kuttenn, 17β-Hydroxysteroid dehydrogenase activity in human breast epithelial cells and fibroblast cultures, Endocrinology 114 (1984) 1483–1489.
[153] M. Poutanen, B. Moncharmont, R. Vihko, 17β-Hydroxysteroid dehydrogenase gene expression in human breast cancer cells: regulation of expression by a progestin, Cancer Res. 52 (1992) 290–294.
[154] P. Couture, C. Theriault, J. Simard, F. Labrie, Androgen receptor-mediated stimulation of 17β-hydroxysteroid dehydrogenase activity by dihydrotestosterone and medroxyprogesterone acetate in ZR-75-1 human breast cancer cells, Endocrinology 132 (1993) 179–185.
[155] M.M. Miettinen, M.V.J. Mustonen, M.H. Poutanen, V. Isomaa, R.K. Vihko, Human 17β-hydroxysteroid dehydrogenase type 1 and type 2 isoenzymes have opposite activities in cultured cells and characteristic cell- and tissue-specific expression, Biochem. J. 314 (1996) 839–845.
[156] M.M. Miettinen, M.H. Poutanen, R.K. Vihko, Characterization of estrogen-dependent growth of cultured MCF-7 human breast-cancer cells expressing 17β-hydroxysteroid-dehydrogenase type 1, Int. J. Cancer 68 (1996) 600–604.
[157] F. Labrie, V. Luu-The, S.-X. Lin, C. Labrie, J. Simard, R. Breton, A. Belanger, The key role of 17β-hydroxysteroid dehydrogenases in sex steroid biology, Steroids 62 (1997) 148–158.
[158] J.R. Pasqualini, G. Chetrite, B.-L. Nguyen, C. Maloche, L. Delalonde, M. Talbi, M.-C. Feinstein, C. Blacker, J. Botella, J. Paris, Estrone sulfate-sulfatase and 17β-hydroxysteroid dehydrogenase activities: a hypothesis for their role in the evolution of human breast cancer from hormone-dependence to hormone-independence, J. Steroid Biochem. Mol. Biol. 53 (1995) 407–412.
[159] S. Fournier, F. Brihmat, J.C. Durand, N. Sterkers, P.M. Martin, F. Kutten, P. Mauvais-Jarvis, Estradiol 17β-hydroxysteroid dehydrogenases, a marker of breast cancer hormone dependency, Cancer Res. 45 (1985) 2895–2899.
[160] T. Suzuki, T. Moriya, N. Ariga, C. Kakeno, M. Kanazawa, H. Sasano, 17β-Hydroxysteroid dehydrogenase type 1 and type 2 in human breast carcinoma: a correlation to clinicopathological parameters, Br. J. Cancer 82 (2000) 518–523.
[161] T. Suzuki, H. Sasano, S. Andersson, J.I. Masson, 3β-Hydroxysteroid

dehydrogenase/Δ5 4-isomerase activity associated with the human 17β-hydroxsteroid dehydrogenase type 2 isoform, J. Clin. Endocrinol. Metab. 85 (2000) 3669–3672.
[162] H. Sasano, T. Susuki, J. Takeyama, H. Utsunomiya, K. Ito, N. Ariga, T. Moriya, 17-Beta-hydroxysteroid dehydrogenase in human breast and endometrial cancer—a new development in intracrinology, Oncology 59 (2000) 5–12 (Suppl.).
[163] N. Ariga, T. Moriya, T. Suzuki, M. Kimura, N. Ohuchi, S. Satomi, H. Sasano, 17β-Hydroxysteroid dehydrogenase type 1 and type 2 in ductal carcinoma in situ and intraductal proliferative lesions of the human breast, Anticancer Res. 20 (2000) 1101–1108.
[164] M. Poutanen, V. Isomaa, K. Kainulainen, R. Vihko, Progestin induction of 17β-hydroxsteroid dehydrogenase enzyme protein in the T-47D human breast-cancer cell line, Int. J. Cancer 46 (1990) 897–901.
[165] H. Peltoketo, V.V. Isomaa, M.H. Poutanen, R. Vihko, Expression and regulation of 17β-hydroxsteroid dehydrogenase type 1, J. Endocrinol. 150 (1996) S21–S30.
[166] N.G. Coldham, V.H.T. James, A possible mechanism for increased breast cell proliferation by progestins through increased reductive 17β-hydroxysteroid dehydrogenase activity, Int. J. Cancer 45 (1990) 174–178.
[167] E.F. Adams, N.G. Coldham, V.H.T. James, Steroidal regulation of oestradiol-17β-dehydrogenase activity of the human breast cancer cell line MCF-7, J. Endocrinol. 118 (1988) 149–154.
[168] C. Malet, A. Vacca, F. Kuttenn, P. Mauvais-Jarvis, 17β-Estradiol dehydrogenase (E2DH) activity in T47D cells, J. Steroid Biochem. Mol. Biol. 39 (1991) 769–775.
[169] G. Chetrite, H.J. Kloosterboer, J.-P. Philippe, J.R. Pasqualini, Effect of org OD14 (Livial®) and its metabolites on 17β-hydroxysteroid dehydrogenase activity in hormone-dependent MCF-7 and T-47D breast cancer cells, Anticancer Res. 19 (1999) 261–268.
[170] T.M. Penning, 17β-Hydroxysteroid dehydrogenase: inhibitors and inhibitor design, Endocr.-Relat. Cancer 3 (1996) 41–56.
[171] M.R. Tremblay, D. Poirier, Overview of a rational approch to design type I 17β-hydroxysteroid dehydrogenase inhibitors without estrogenic activity: chemical synthesis and biological evaluation, J. Steroid Biochem. Mol. Biol. 66 (1998) 179–191.
[172] J.D. Pelletier, F. Labrie, D. Poirier, *N*-butyl, *N*-methyl, 11[3′,17′((dihydroxy)-1′,3′,5′(10′)-estratrien-16′(-yl]-9(R/S)-bromoundecanamide: synthesis and 17β-HSD inhibiting, estrogenic and antestrogenic activities, Steroids 59 (1994) 536–547.
[173] M.W. Sawicki, M. Erman, T. Puranen, P. Vihko, D. Ghosh, Structure of the ternary complex of human 17β-hydroxysteroid dehydrogenase type 1 with 3-hydroxyestra-1,3,5,7-tetraen-17-one (equilin) and NADP+, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 840–845.
[174] C. Gunnarsson, K. Kirschner, E. Hellqvist, O. Stal, Expression of 17β-hydroxysteroid dehydrogenases and correlation to prognosis in postmenopausal breast cancer patients, Breast Cancer Res. Treat. 82 (Suppl. 1) (2003) S111 (Abst. 459).
[175] A.M.H. Brodie, Aromatase inhibitors and their application to the treatment of breast cancer, in: J.R. Pasqualini (Ed.), Breast Cancer, Prognosis, Treatment, and Prevention, Marcel Dekker, New York, 2002, pp. 251–269.
[176] Aromatase 2002, in: A. Brodie, M. Dowsett, N. Harada, P. Lonning, B. Miller, J.R. Pasqualini, R. Santen, H. Sasano, E. Simpson (Eds.), Proceedings of the VI International Aromatase Conference, J. Steroid Biochem. Mol. Biol., vol. 86, 2003, pp. 219–507.
[177] D.M. Strickland, R.D. Gambrell, C.A. Butzin, K. Strickland, The relationship between breast cancer survival and prior postmenopausal estrogen use, Obstet. Gynaecol. 80 (1992) 400–404.
[178] D.B. Willis, E.E. Calle, H.L. Miracle-McMahill, C.W. Heath Jr., Estrogen replacement therapy and risk of fatal breast cancer in a prospective cohort of postmenopausal women in the United States, Cancer Causes Control 7 (1996) 449–457.
[179] I. Persson, J. Yuen, L. Bergvist, C. Schairer, Cancer incidence and mortality in women receiving estrogen and estrogen-progestin replacement therapy—long-term follow-up of a Swedish cohort, Int. J. Cancer 67 (1996) 327–332.
[180] P. Bonnier, F. Bessenay, A.J. Sasco, B. Beedassy, C. Lejeune, S. Romain, C. Charpin, L. Piana, P.M. Martin, Impact of menopausal hormone-replacement therapy on clinical and laboratory characteristics of breast cancer, Int. J. Cancer 79 (1998) 278–282.
[181] H. Jernström, J. Frenander, M. Fernö, H. Olsson, Hormone replacement therapy before breast cancer diagnosis significantly reduces the overall death rate compared with never-use among 984 breast cancer patients, Br. J. Cancer 80 (1999) 1453–1458.
[182] H.P. Schneider, C. Jackisch, Potential benefits of estrogens and progestogens on breast cancer, Int. J. Fert. Women's Med. 43 (1998) 278–285.