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Estrone sulfatase versus estrone sulfotransferase in human breast cancer: potential clinical applications ☆

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Abstract

Estrone sulfate (E_1S) is concentrated in high levels in human breast cancer tissue. The values are particularly high in postmenopausal women and many times those circulating in the plasma. Also, the tissular concentration of this conjugate are significantly higher in tumoural tissue than in the area of the breast considered as normal. The enzyme which hydrolyzes E_1S: sulfatase, as well as the enzyme which biosynthesises this conjugate: sulfotransferase, are present in significant concentrations in breast cancer tissue. Consequently, E_1S is a balance between the activities of the two enzymes. As breast cancer tissue has all the enzymes necessary for the synthesis of estradiol (E_2), and the formation of E_2 from E_1S 'via sulfatase' is the main pathway, it was very attractive to explore inhibitory agents of this enzyme. It was observed that different substances including antiestrogens (4-hydroxytamoxifen, ICI 164,384) and various progestins (promegestone, nomegestrol acetate, medrogestone) as well as Org OD14 (tibolone) can block the sulfatase activity. In addition, it was demonstrated that different progestins (medrogestone, nomegestrol acetate, TX-525) and org OD14 can stimulate the sulfotransferase activity for the formation of the biologically inactive E_1S. It is concluded that the inhibition of sulfatase and the stimulation of sulfotransferase activity can open interesting possibilities to explore these effects in patients with breast cancer.

1. Introduction

Breast cancer is one of the major causes of cancer-related death among women, and recent statistical information indicates that in the United States one woman in eight will develop this disease during their lifetime; the values are one in 12 for countries of the European Community and one in 80 for Japan. It is now well established that increased exposure to estradiol (E_2) is an important risk factor for the genesis and evolution of breast tumours, and most of them (approximately 95–97%) in their early stage are estrogen-sensitive [1–4]. 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 estrone (E_1), 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 tumoural biosynthesis and accumulation of these hormones [5–8].

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 [9].

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 [10–12], and the 'sulfatase pathway' which converts estrone sulfate (E_1S) into E_1 by the estrone-sulfatase (EC:3.1.6.1) [13–17]. The final step of steroidogenesis is the conversion of the weak E_1 to the

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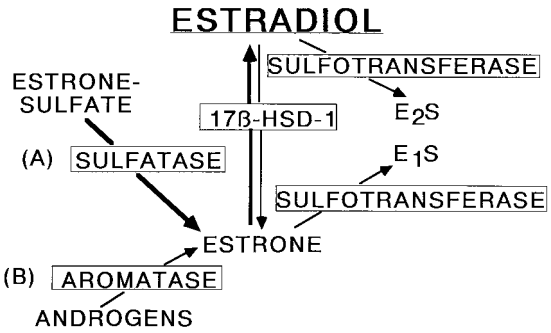

Fig. 1. Enzymatic mechanisms involved in the formation and transformation of estrogens in human breast cancer. The sulfatase pathway (a) is quantitatively 100–500 times higher than that of the aromatase pathway (b). 17β-HSD-1 is 17β-hydroxysteroid dehydrogenase (type 1); E_1S is estrone-3-sulfate and E_2S estradiol sulfate.

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) [18–20].

Quantitative evaluation indicates that in human breast tumour E_1S 'via sulfatase' is a much more likely precursor for E_2 than is androstenedione 'via aromatase' [21].

It is also well established that steroid sulfotransferases (ST), which convert estrogens into their sulfates, are also present in breast cancer tissues [22–25].

Fig. 1 gives a general view of estrogen formation and transformation in human breast cancer.

2. Importance of estrone sulfate concentration in breast cancer

The sulfoconjugation of estrogens is an important feature to protect breast and endometrial tissues, as well as fetal target tissues, since this metabolism can regulate the level of active E_2 [26,27].

Estrone sulfate (E_1S) is quantitatively the most important form of circulating estrogens in both cycling and postmenopausal women and their concentrations are 5–10 times those of unconjugated estrogens [28–31]. The water-soluble structure of E_1S allows a higher binding to serum proteins and a clearance from the blood compartment two orders of magnitude more slowly than the unconjugated forms. The importance of E_1S in the breast tumour is, first because the high concentration of sulfoconjugates creates a reservoir of precursors for the biosynthesis of biologically active E_2 through the action of endogenous sulfatase, and second because sulfoconjugates are biologically inactive, as the presence of the charged sulfonate group prevents the binding of this estrogen to its receptor (ER).

3. Control of estrone sulfatase in breast cancer

Sulfonation is a well known mechanism by which the target organs convert different potentially active compounds, such as steroid hormones, bile acid, neurotransmitters, proteoglycans, drugs or carcinogens, into biologically inactive hydrophilic conjugates.

The formation of estrogen sulfates (ES) (sulfotransferases) in breast cancer or in other tissues can be controlled by the reverse reaction (sulfatase), consequently the tissular levels of ES are the result of a balance between the two enzymes: estrone sulfatase and estrone sulfotransferase.

For many years the endocrine therapy in breast cancer has been mainly by the utilization of antiestrogens, which block the estrogen receptor. Treatment with the antiestrogen tamoxifen (Nolvadex: tamoxifen citrate) to millions of women with breast cancer has had a benefit of 30–35% free of symptoms of the disease and a 20–25% reduction of mortality. More recently, another endocrine therapy has been explored by inhibiting the tissular estradiol production using different anti-enzyme agents involved in the biosynthesis of this hormone. At present, the positive effect of anti-aromatase compounds on the benefit in breast cancer patients is well documented [32–35]. However, as E_1S in human breast cancer is quantitatively the most important precursor of E_2, new possibilities can be opened to block E_2 which is originated through his conjugate via the 'sulfatase pathway'.

3.1. Inhibitory agents of estrone sulfate-sulfatase activity in breast cancer

Estrone sulfate-sulfatase belongs to class C of the aryl sulfatase family, and the most intense activity detected in breast tumour tissue or breast cancer cells is present in the mitochondrial/microsomal subcellular fraction [13,36]. Recently, the presence of a nuclear isozyme has been detected in the liver of female rats. This isozyme shows different biochemical properties (e.g. higher affinity for E_1S) to the microsomal sulfatase [37].

In human hormone-dependent breast cancer cells (MCF-7, T-47D), the estrone sulfatase activity is high, as well as in intact cells or in homogenates. In contrast, hormone-independent breast cancer cells (MDA-MB-231, MDA-MB-468) show very low sulfatase activity in intact cells, but the activity is restored when the cells are homogenized [38,39]. The mRNA of the sulfatase are present in both the hormone-dependent and -independent breast cancer cells and the expression of this mRNA correlated with the sulfatase activity [40].

The data give clear evidence that the sulfatases are present in the hormone-independent cells, but do not

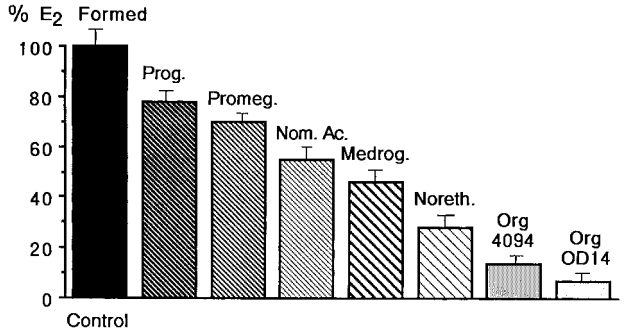

Fig. 2. Comparative effects of progestins on the inhibition of the conversion of estrone sulfate to estradiol in the T-47D breast cancer cells. Preconfluent cells were incubated for 24h at 37°C with 5×10^{-9} mol/l [^{3}H]-estrone sulfate (physiological concentration), alone or in the presence of progestins at the concentration of 5×10^{-7} mol/l. Qualitative and quantitative analyses of E_2 in the cell compartment were performed by the thin-layer chromatography method. Results (pmol of E_2 formed/mg DNA) are expressed in percent (%) of control values considered as 100%. The data are the means ± S.E.M. of duplicate determinations of three to six experiments. Prog. means progesterone; Promeg. promegestone (R-5020), Nom. Ac. nomegestrol acetate; Medrog. medrogestone; Noreth. norethisterone; Org 4094 3α-hydroxy derivative of Org OD14; and Org OD14 Tibolone, active substance of Livial®.

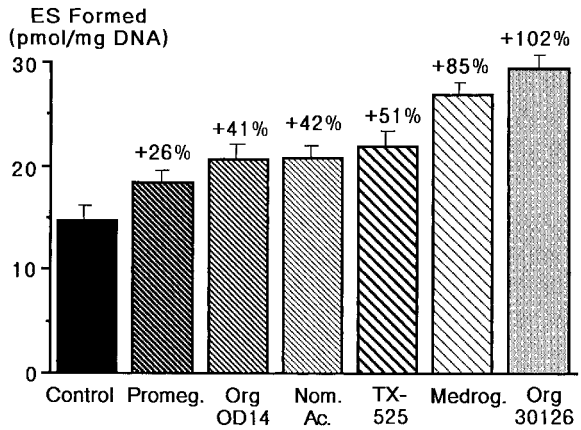

Fig. 3. Comparative effects of progestins on the conversion of estrone to estrogen sulfates in the T-47D breast cancer cells. Preconfluent cells were incubated for 24 h at 37°C with a physiological concentration of estrone ([^{3}H]-E_1: 5×10^{-9} mol/l) alone (control: nontreated cells) or in the presence of progestins at the concentration of 5×10^{-8} mol/l. Qualitative and quantitative analyses of estrogen sulfates (ES) in the culture medium were performed by the thin-layer chromatography method. Results (pmol of ES formed/mg DNA) are expressed in percent (%) of control values. The data are the means ± S.E.M. of duplicate determinations of three to six independent experiments. Promeg. means promegestone (R-5020); Org OD14 Tibolone, active substance of Livial®; Nom. Ac. nomegestrol acetate; TX-525 a 19-nor progestin of Theramex Laboratories; Medrog. medrogestone; Org 30126 3β-hydroxy derivative of Org OD14.

operate in the complete cells. What is the reason that, in spite of the existence of the enzyme, very little E_1S is hydrolyzed with these intact cells? The reply to this question is not clear at present, but we suggest the presence of repressive factor(s) or its sequestering in an inactive form for this kind of cell. More information is needed to elucidate this mechanism.

3.1.1. Effect of anti-estrogens

Beside the classical effect of anti-estrogens on the estrogen receptor, these agents show anti-sulfatase activity. Tamoxifen, 4-hydroxytamoxifen and the pure anti-estrogen ICI 164,384 at concentrations of 10^{-6}–10^{-5} M have an inhibitory effect on the conversion of physiological concentrations (5×10^{-9} M) of E_1S to E_2 in hormone-dependent breast cancer cells [41–45].

3.1.2. Effect of progestins

Various progesterone derivatives (e.g. medrogestone), as well as norprogestins (e.g. nomegestrol acetate, Org OD14 (tibolone), promegestone) provoke a significant decrease of E_2 formation when physiological concentrations of E_1S are incubated with breast cancer cells (MCF-7 and T-47D) [46–49]. Fig. 2 shows the inhibitory effect of different progestins in the conversion of E_1S to E_2 in the hormone-dependent breast cancer cells.

3.1.3. Effect of other compounds

Interesting information as a potent anti-sulfatase agent was obtained with EMATE (estrone-3-*0*-sulfamate) and related compounds [50]. The sulfatase inhibitory effect of EMATE was obtained in in vitro [51] as well as in vivo [52] studies.

4. Control of sulfotransferase activity in breast cancer

Another interesting approach in the control of estradiol in breast cancer tissue is to increase the conversion of estrogens to the inactive form by sulfonation (sulfotransferase activity).

The superfamily of sulfotransferases (ST) includes three categories of isozymes with distinct but overlapping substrate specificity. Estrogen-ST (EST; E.C.: 2.8.2.4.), hydroxy-ST (HST) (e.g. dehydroepiandrosterone-ST) and phenol-ST (PST) (e.g. aryl-ST), which are divided into a phenol sulfating form (P-PST) and a monoamine sulfating form (M-PST) [53–55]. Sulfonation of E_1 is specifically for EST at nanomolar concentrations, whereas P-PST and HST can also act on estrogens but at micromolar concentrations [56,57].

EST is a soluble cytosolic enzyme, presumably dimeric, with multiple isoform charges and difficult to purify [58,59]. However, EST cDNA of various origins have been cloned and sequenced, as well as a gene of hEST and PST, showing a great homology between them. There is some contradiction as to whether ster-

oid sulfotransferases are correlated with receptor status (ER/PR) in breast tumours [60–62].

Recent data have shown that some progestins (Org OD14 and its main metabolites Org 30126 and Org 4094, medrogestone, nomegestrol acetate, TX-525 or promegestone) at low concentrations (5×10^{-8}–5×10^{-7} mol/l) have the capacity to increase the sulfotransferase activities in hormone-dependent MCF-7 and T-47D breast cancer cell lines [49,63,64] (see Fig. 3). The mechanism by which progestins modify enzymatic activities in breast cancer tissues appears to be complex. In addition, in a recent paper, it was demonstrated that the rate of estrogen-ST activity can be correlated with the expression of human EST1 mRNA (derived from STM gene according to HUGO nomenclature) in hormone-dependent and hormone-independent breast cancer cell lines [64].

5. Conclusions and perspectives

The findings in this laboratory and others demonstrate very clearly that human breast cancer tissue contains the enzymes necessary for the formation of estrogens; this includes sulfatase, aromatase and 17β-hydroxysteroid dehydrogenase. Sulfotransferases, which transform estrogens into their sulfates, are also present in this tissue.

The information that in postmenopausal women the concentrations of the various estrogens, in particular estrone sulfate (E_1S), in cancer tissues are many times higher than those found in plasma suggests that this tissue can accumulate these steroids. Also, recent data show that the concentration of E_1S is significantly higher in the carcinoma tissue than in the area of the breast considered as normal. The data on the high concentration of estrogens in the breast cancer tissues is particularly pertinent during the postmenopausal period in which it is well known that the levels of circulating estrogen are very low. The data suggest a local production of these hormones in the breast cancer tissue itself.

In recent years, one of the major goals in breast cancer research was to elucidate the mechanism and discover new drugs which can block estrogen production. A very big advance has been made using anti-aromatase agents. As E_1S is quantitatively the most important precursor of E_2, also new possibilities can be opened to block E_2 which is originated through this conjugate.

The present data demonstrate that in the hormone-dependent breast cancer cells, anti-estrogens, (4-hydroxytamoxifen; ICI 164,384), the progestins: medrogestone, Org OD14 (tibolone), nomegestrol acetate, promegestone, can inhibit the activity of two enzymes: sulfatase, as well as 17β-hydroxysteroid dehydrogenase, which are involved in the last steps of E_2 biosynthesis and that the same compounds: nomegestrol acetate, medrogestone or Org OD14, at low doses can stimulate the sulfotransferase which are involved in the formation of the inactive sulfates, can open new possibilities for clinical trials in breast cancer patients and consequently for new therapeutic possibilities for this disease.

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