Correlation of Estrogen Sulfotransferase Activity and Proliferation in Normal and Carcinomatous Human Breast. A Hypothesis

JORGE RAUL PASQUALINI[1] and GERARD SAMUEL CHETRITE[2]

[1]*Hormones and Cancer Research Unit, Institut de Puériculture et de Périnatalogie, 26 Boulevard Brune, 75014 Paris;*
[2]*Assistance Publique – Hôpitaux de Paris, Centre d'Investigations Biomédicales Paris-Sud, CHU Bicêtre, and INSERM U693, Faculté de Médecine Paris-Sud, 94276 Le Kremlin-Bicêtre, France*

Abstract. *Background: Sulfotransferases are present in normal and cancerous human breast tissues. The purpose of this article is to present a hypothetical correlation of sulfotransferase activity with proliferation in breast cancer. Materials and Methods: Sulfotransferases were evaluated in breast cancer cells by determining the transformation of non-conjugated estrogens to the sulfates. Proliferation was evaluated by the action on cell growth or the size of a transplanted tumor. The effect was obtained using the progestins: nomegestrol acetate, promegestone, and medrogestone, as well as tibolone and its metabolites at concentrations of $5x10^{-5}$ to $5x10^{-9}$ M. Results: A possible correlation of sulfotransferase activity stimulation and cell growth inhibition provoked by the various progestins used, or by tibolone and its metabolites was shown. Conclusion: It is suggested that the antiproliferative effect of these compounds could be related to the decrease of bioactive estradiol by the formation of its biologically inactive sulfate as a consequence of the stimulatory effect by the various progestins or tibolone on sulfotransferase activity.*

I. Introduction

There is substantial information that human breast tissue contains all the enzymes necessary for the local bioformation of estradiol (E_2) from circulating precursors. Two principal pathways are implicated in the last steps of E_2 formation in breast tissue: the 'sulfatase pathway' in which estrone sulfatase (EC: 3.1.6.2) converts estrone sulfate (E_1S) into estrone (E_1) and the 'aromatase pathway' which transforms androgens into estrogens. The final step of steroidogenesis is the conversion of the weak E_1 into the potent biologically active E_2 *via* reductive 17β-hydroxysteroid dehydrogenase type 1 activity (17β-HSD-1) (EC: 1.1.1.62). It is also well established that breast tissue contains steroid sulfotransferase (EC: 2.8.2.4), an enzyme which converts estrogens into the biologically inactive sulfate by transfer of a sulfuryl group (SO_3) from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) cofactor. Figure 1 gives an overview of estrogen formation and transformation in the human breast.

The presence of estrogen sulfotransferase in human breast tissue is well documented (1-5). Sulfotransferases comprise a series of superfamilies which include two major subfamilies: i) the phenol sulfotransferase family, designated as SULTs: 1A1, 1A2, 1A3, 1B1, 1C2, 1C4, and the estrogen sulfotransferase, SULT1E1; and ii) the hydroxysteroid SULTs, which include dehydroepiandrosterone sulfotransferase (SULT2A1) and the two SULTB1 forms that sulfonate cholesterol, pregnenolone and other steroids (6).

The ability of SULT1E1 to sulfonate estrogens at physiological concentrations is important in regulating their activation of estrogen receptors in target tissues of this hormone.

II. Estrogen sulfotransferases in the human breast

A) In the normal breast. The presence of estrone sulfotransferase (SULT1E1 or EST) was detected in a normal breast cell line, the Huma 7, obtained from reduction mammoplasty by Wild *et al.* (3). These authors observed that SULT1E1 activity in this cell line far exceeded that in either MCF-7 or ZR-75-1 breast cancer cells. Comparative studies indicated that after 24 h incubation, 50% of estrogens are sulfonated in normal cells and only 10% in the malignant cells. Anderson and Howell (7) using two normal breast epithelial cells, the MTSV 1-7 and the MRSV 4-4 produced by simian virus 40 immortalization

Correspondence to: Dr. Jorge Raul Pasqualini, Hormones and Cancer Research Unit, Institut de Puériculture et de Périnatalogie, 26 Boulevard Brune, 75014 Paris, France. Tel: +33 1 45424121/ 45399109, Fax: +33 1 45426121, e-mail: jorge.pasqualini@wanadoo.fr

Key Words: Breast cancer, estrogen sulfotransferase, proliferation.

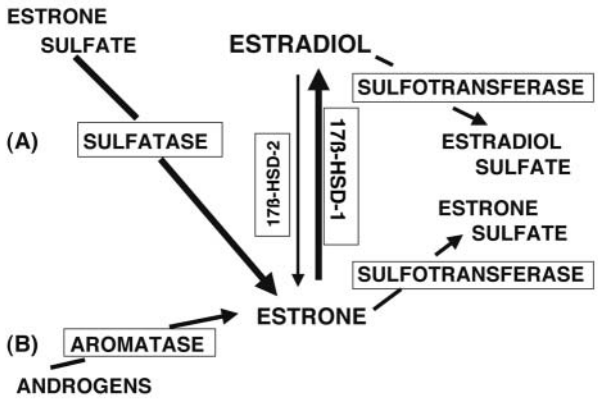

Figure 1. *The enzymatic mechanism involved in the formation and transformation of estrogens in human breast cancer. The sulfatase pathway (A) is quantitatively 100 to 500 times greater than that of the aromatase pathway (B). 17β-HSD-1: 17β-hydroxysteroid dehydrogenase type 1. 17β-HSD-2: 17β-hydroxysteroid dehydrogenase type 2.*

cells obtained from human milk, confirmed the presence of SULT1E1. In these normal breast cells, SULT1E1 has an affinity for E_2 sulfation in the nanomolar concentration range (8-10). Consequently, SULT1E1 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 estrogen receptor. This ability of SULT1E1 to render estrogens physiologically inactive *via* sulfoconjugation could be related to breast cell proliferation (see section IV).

B) In breast cancer. Sulfotransferase activity in either breast cancer tissues or cells has been found by different authors, but there are discrepancies concerning the type of SULT; some authors observed significant amounts of phenol sulfotransferase but only trace levels of hydroxysteroid and estrogen sulfotransferase in several hormone-dependent breast cancer cells (8, 11), while others reported the presence of these sulfotransferases in MCF-7, ZR-75 cells and breast tumors (12, 13).

Falany and Falany (8) suggested that in breast cancer cells the sulfoconjugated activity is mainly due to the human phenol sulfotransferase, SULT1A1, an enzyme that is more efficient with estrogens at micromolar than at nanomolar concentrations. SULT1E1 has an activity for estrogen sulfation 300-fold higher than SULT1A1 (14).

III. Physiological importance of sulfotransferases in normal and in carcinomatous breast cells

Normal HME breast cells contain SULT1E1 at physiological concentrations which is not present in MCF-7 nor in some other breast cancer cells (*e.g.*: ZR75-1, T-47D, BT-20, MDA-MB-231) (8, 11). The data indicate that estrogens (estrone or estradiol) are sulfonated in breast cancer cells by phenol sulfotransferase SULT1A1, an enzyme that only acts preferentially at micromolar concentrations of estrogens. The loss of SULT1A1 expression during the process of breast cancerization may be of importance because this enzyme inactivates estradiol, suggesting that the inability of the breast cell to block E_2 could be an important mechanism contributing to abnormal growth of the breast cells by controlling the E_2 levels.

In a study which compared the sulfotransferase activities of the normal human mammary epithelial (HME) and the MCF-7 breast cells, it was observed that after incubation with 20 nM of E_2, the level of sulfonated E_2 detected in the HME medium was 10 times that found in the medium of MCF-7 cells (8) indicating that HME cells secreted estradiol sulfate into the medium at a significantly higher rate than did MCF-7 breast cancer cells.

As estrogen sulfates do not bind to the estrogen receptor, factors that altered the sulfotransferase activities, and which can consequently affect estrogen transformation, may be of importance in controlling hormone-dependent cellular growth, suggesting that in normal breast tissue estrogen stimulation of growth and differentiation is specifically controlled, contrasting markedly with the abnormal proliferation of breast cancer cells (15).

In order to explore the possibility that estrogen sulfotransferase activity disappears during the process of breast cancerization, Falany and Falany (8, 16) transfected MCF-7 cells with a SULT1E1 expression vector and observed that after incubation with 20 nM of E_2, sulfation occured significantly more rapidly with the transfected MCF-7 cells than with the control cells. Qian *et al.* (17) confirmed this data after evaluation of the physiological significance of SULT1E1 expression by cDNA transfection using MCF-7 cells and observed that in these transformed cells the response to physiological concentrations of E_2 was reduced by up to 70%, as determined in a responsive reporter gene assay.

IV. Effect of various progestins and of tibolone and its metabolites on breast cell proliferation

The biological effects of progestins can be influenced by various factors: their structure, the biological responses considered, their affinity for the receptor or other steroid receptors, experimental conditions, the dose concentrations, or their metabolic transformations. We indicate here the effect on cell growth of various progestins, as well as of tibolone.

A) Promegestone. Promegestone (R-5020, Surgestone®) is a 19-norprogesterone derivative largely used in the treatment of premenopausal complaints. Using normal human breast

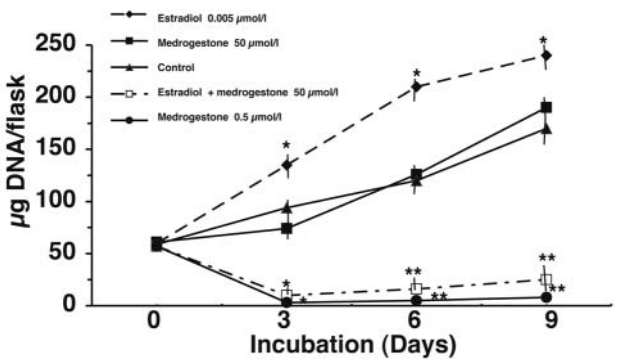

Figure 2. *Effect of medrogestone alone or in the presence of estradiol (E_2) on proliferation of MCF-7 breast cancer cells. MCF-7 cells were grown in 75 cm^2 flasks in MEM culture medium containing 5% FCS treated with dextran-coated charcoal. The cells were treated with medrogestone alone ($5x10^{-5}$ M, $5x10^{-7}$ M), E2 alone ($5x10^{-9}$ M), or medrogestone ($5x10^{-5}$ M) + E_2 ($5x10^{-9}$ M) (day 0). Control corresponds to non-treated cells. DNA content was evaluated after 3, 6, and 9 days of culture (unpublished data). *p≤0.05 vs. control value; **p≤0.01 vs. control value.*

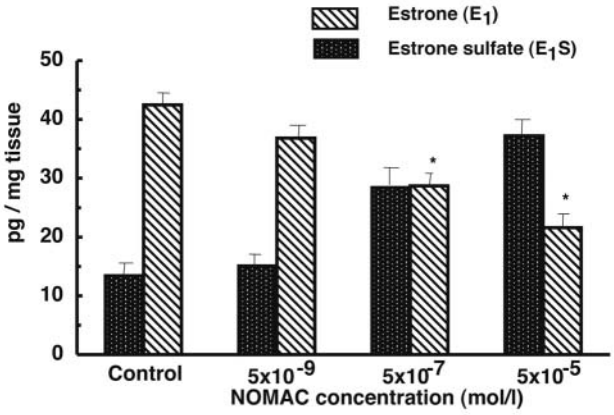

Figure 3. *Effects of nomegestrol acetate (NOMAC) on the conversion of estrone sulfate (E_1S) to estrone (E_1) in the human cancer breast tissues. Slices of cancer breast tissues (50-65 mg) were incubated for 4 h at 37°C with a physiological concentration of estrone sulfate ([^{3}H]-E_1S: $5x10^{-9}$ M) alone (control: non-treated cells) or in the presence of NOMAC at a concentration of $5x10^{-9}$ M to $5x10^{-5}$ M. Estrogens were quantified after isolation of the hormones by thin layer chromatography. The data are the mean±SEM of duplicate determinations of three independent experiments. *p≤0.05 vs. E_1 control values (from ref. 24).*

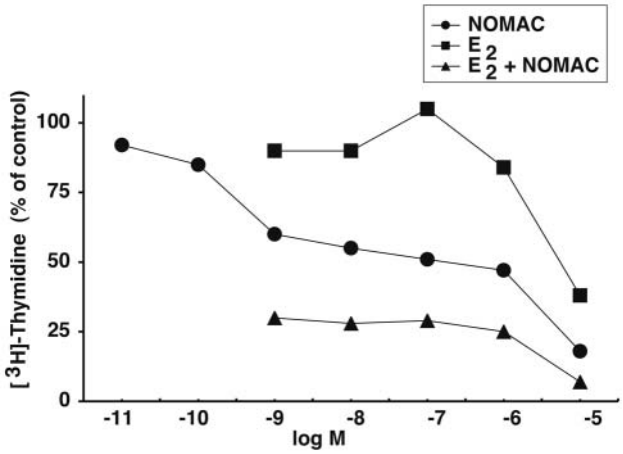

Figure 4. *Effects of nomegestrol acetate (NOMAC) and estradiol (E_2) on the growth of T-47D breast cancer cells: concentration-response curves. Exponentially growing T-47D cells were plated in 24- or 48-well dishes of DMEM medium containing 5% fetal calf serum (FCS) in the presence of insulin. After 1 day, cells were treated with NOMAC alone, E_2 alone, or NOMAC + E_2 $1x10^{-9}$ M. Media were replaced 3 days later and after an additional 3-day period the cells were processed for a [^{3}H]-thymidine incorporation assay. Results are expressed as % of control [^{3}H]-thymidine values (without NOMAC and without E_2) (from ref. 25).*

epithelial cells, it was demonstrated that promegestone can decrease cell proliferation (18-20). These authors also found that progestins can inhibit the proliferative effect provoked by E_2; however, McManus and Welsch (21) and Longman and Buehring (22) observed no effect.

B) Effect of medrogestone. Medrogestone is a synthetic pregnane derivative used in the treatment of pathological deficiency of natural progesterone. This compound produces secretory activity in the estrogen-primed uterus, is thermogenic and acts as an antiestrogen and antigonadotropin.

In a series of studies, it was demonstrated that medrogestone can inhibit the proliferation of T-47D breast cancer cells, as well as the proliferative effect provoked by E_2 (unpublished data) (Figure 2).

C) Effect of nomegestrol acetate. Nomegestrol acetate (NOMAC), a 17α-hydroxy-nor-progesterone derivative (the active substance in Lutenyl®), is a potent and useful clinical synthetic progestin for the treatment of menopausal complaints and is under current development for oral contraception. This compound does not possess estrogenic activity and it was demonstrated that it can block the sulfatase and 17β-hydroxysteroid dehydrogenase activities in breast cancer cells (23) and in the whole breast tissue (24) (Figure 3).

Various studies have shown that it can have anti-proliferative effects in MCF-7 and T-47D breast cancer cells (25, 26) (Figure 4).

D) Effect of tibolone and its metabolites. Tibolone (Org OD14, active substance of Livial®) is a 19-nortestosterone derivative with weak estrogenic, androgenic and progestagenic properties used to prevent climacteric symptoms and postmenopausal osteoporosis as well as vasomotor or vaginal symptoms. It is rapidly metabolized to its main active metabolites, including 3α- and 3β-hydroxy-

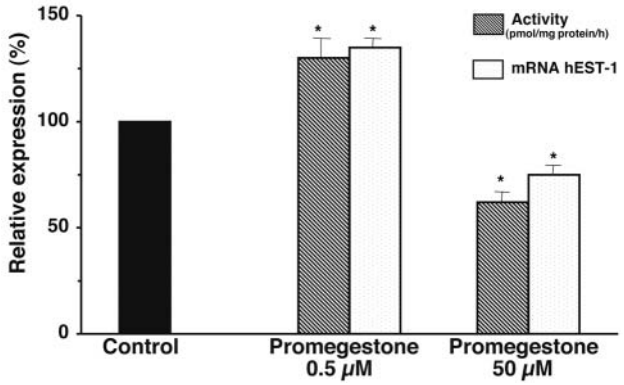

Figure 5. *Effects of the progestin promegestone on sulfotransferase activity and mRNA expression of estrogen sulfotransferase type 1 (hEST-1) in the hormone-dependent T-47D human breast cancer cell line. Relative expression of the mRNA (using RT-PCR amplification) and the activity (in pmol/mg protein/h) of hEST in T-47D cells non-treated (control) or treated with promegestone at a concentration of $5x10^{-5}$ or $5x10^{-7}$ M. The control value is assigned 100%. The data represent the mean±S.E.M. of 2-3 experiments (from ref. 30). *p≤0.05 vs. control value;*

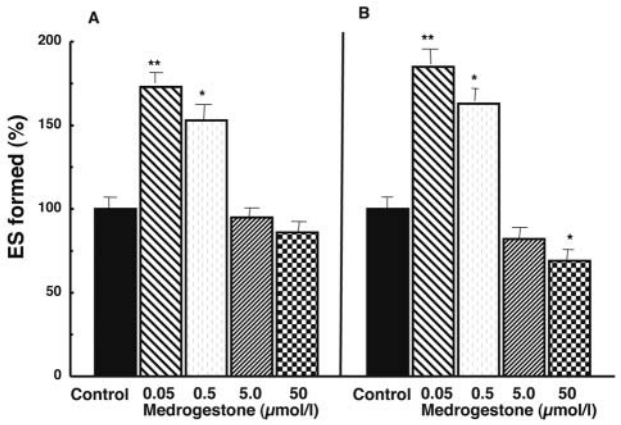

Figure 6. *Effects of the progestin medrogestone on the conversion of estrone (E_1) to estrogen sulfates (ES) in (A) hormone-dependent MCF-7 cells and (B) T-47D human breast cancer cells. Results (pmol of ES formed/mg DNA) are expressed as % of control values. The data are the mean±SEM of duplicate determinations of three independent experiments. *p≤0.05 vs. control value (nontreated cells). **p≤0.01 vs. control value (non-treated cells) (from ref. 31).*

tibolone and 4-ene-tibolone, which could explain the tissue-specific effect of tibolone (27). In a series of studies it was demonstrated that tibolone inhibited the growth of tumors in DMBA-treated rats. In addition, this compound increases apoptosis and decreases cell proliferation (28, 29).

V. Modulation of sulfotransferase activity in breast cancer cells

In mammary tissues, SULT1E1 has been implicated in estrogen inactivation by catalyzing the sulfonation of estrogens (E_1 and E_2). This is of biological importance since the affinity of SULT1E1 for E_2 is analogous to that of E_2 for its receptor (Kd at nanomolar concentrations) and corresponds to the physiological tissue concentration of the hormone. Thus, the modulation of SULT1E1 activity is an important factor in controlling the amount of biologically active unconjugated E_2 in the breast tissue. Various compounds have been tested, in particular some progestins (nomegestrol acetate, medrogestone, promegestone) as well as tibolone and its main metabolites (3α- and 3β-hydroxy derivatives, 4-ene isomer), for their capacity to modulate sulfotransferase activity in hormone-dependent breast cancer cell lines, MCF-7 and T-47D.

A) Effect of progestins on sulfotransferase activity.

(i) Promegestone. When this progestin was incubated with [^{3}H]-estrone ($5x10^{-9}$ M) and MCF-7 and T-47D breast cancer cells in culture, a biphasic effect was observed on the sulfotransferase activity. At a low concentration ($5x10^{-7}$ M), promegestone stimulated the formation of estrogen sulfates in both cell lines (+26%), whereas at a high concentration ($5x10^{-5}$ M) the sulfotransferase activity was inhibited (-41%) (30). This dose-dependent dual effect on the catalytic activity of sulfotransferase could be correlated with the expression of the mRNA hEST1 in these two cell lines. At a concentration of $5x10^{-7}$ M of promegestone, the mRNA hEST1 expression was increased by 35% and 75% in T-47D and MCF-7 cells, respectively. In contrast, a concentration of $5x10^{-5}$ M reduced the level of mRNA hEST1 expression by 25% and 36% in T-47D and MCF-7 cells, respectively (30) (Figure 5).

(ii) Medrogestone. Medrogestone, a synthetic progesterone derivative, also has a biphasic action on the sulfotransferase activity. At low concentrations ($5x10^{-8}$ to $5x10^{-7}$ M), medrogestone stimulated the formation of estrogen sulfates by 73% and 53% respectively for MCF-7 cells and by 84% and 63% for T-47D cells, whereas at high concentrations ($5x10^{-6}$ to $5x10^{-5}$ M) the sulfotransferase activity was not modified in MCF-7 cells and was inhibited by 31% at $5x10^{-5}$ M only in T-47D cells (31). The effect of medrogestone on the sulfotransferase activity in MCF-7 and T-47D breast cancer cells is indicated in Figure 6.

(iii) Nomegestrol acetate (NOMAC). It was also observed that NOMAC significantly stimulated the sulfotransferase activity at concentrations of $5x10^{-8}$, $5x10^{-7}$, and $5x10^{-6}$ M by 61%, 83%, and 49%, respectively, in MCF-7 cells, and by 41%, 69%, and 58%, respectively, in T-47D cells (32) (Figure 7). At a high concentration ($5x10^{-5}$ M) NOMAC had no significant effect on sulfotransferase activity in relation to control values of either cell line.

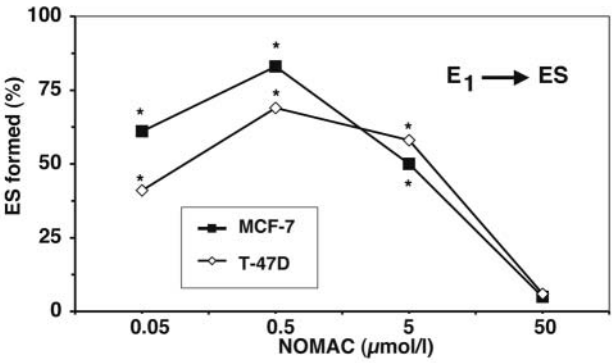

Figure 7. *Effects of nomegestrol acetate (NOMAC) on the conversion of estrone (E_1) to estrogen sulfates (ES) in the hormone-dependent MCF-7 and T-47D human breast cancer cell lines. Preconfluent cells were incubated for 24 h at 37˚C with a physiological concentration of estrone ([^{3}H]-E_1: 5x10^{-9} M) alone (control: non-treated cells) or in the presence of nomegestrol acetate at a concentration of 5x10^{-8} M to 5x10^{-5} M. Estrogen sulfates were quantified after isolation of the hormones in the culture medium by thin layer chromatography. The percentage of ES formed was obtained by calculating the ratio: [(test-control) / control] x 100. The data are the mean±SEM of duplicate determinations of 2-3 independent experiments. *p≤0.05 vs. control values (from ref. 32).*

B) Effect of tibolone and its main metabolites on sulfotransferase activity.

In relation to the sulfotransferase activity in hormone-dependent breast cancer cells, tibolone had a dual effect on the formation of estrogen sulfates. At low concentrations (5x10^{-8} to 5x10^{-7} M), tibolone and its 3α- and 3β-hydroxy derivatives significantly increased the sulfotransferase activity in both cell lines. In MCF-7 cells, the values were 63% and 44% respectively for tibolone, 50% and 37% respectively for 3α-OH-metabolites, and 101% and 74% respectively for 3β-OH-metabolites. At 5x10^{-6} M, no effect was observed for these three compounds, whereas at 5x10^{-5} M tibolone and its 3α- and 3β-metabolites inhibited sulfotransferase activity by 64%, 63%, and 54%, respectively. The 4-ene isomer had no effect at 5x10^{-8}, 5x10^{-7}, or 5x10^{-6} M, but decreased sulfotransferase activity at 5x10^{-5} M by 59% (Figure 8). A comparable effect was observed with T-47D cells (33).

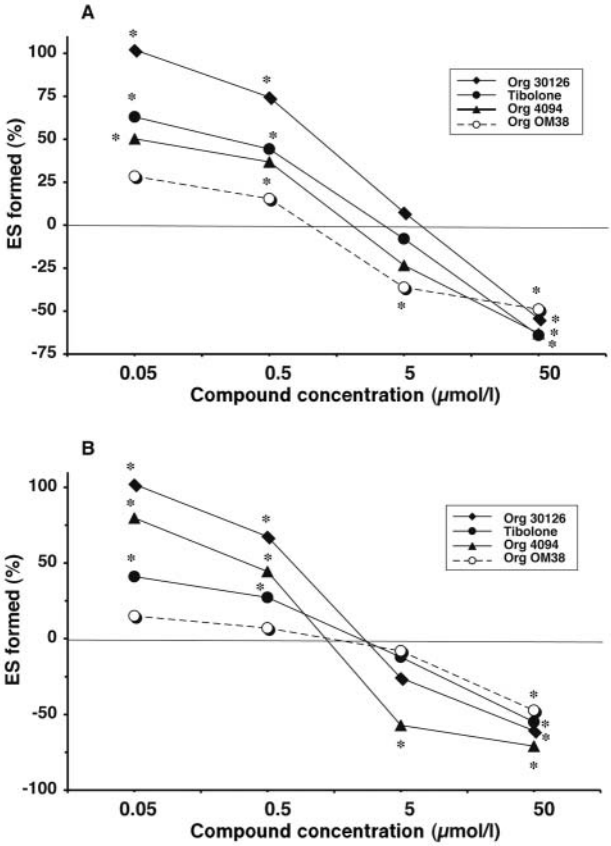

Figure 8. *Comparative effects of tibolone (Org OD14) and of its main metabolites on the conversion of estrone (E_1) to estrogen sulfates (ES) in (A) the hormone-dependent MCF-7 and (B) T-47D human breast cancer cell lines. Results (pmol of ES formed in culture medium /mg DNA) are expressed as a percentage (%) of the control value, considered as 100%. The data represent the mean±SEM of duplicate determinations of 3 to 4 independent experiments. Org OD14: tibolone; Org 30126: 3β-OH metabolite of tibolone; Org 4094: 3α-OH metabolite of tibolone; Org OM38: 4-en isomer of tibolone. *p≤0.05 vs. control value [^{3}H]-E_1: 5x10^{-9} M alone (from ref. 33).*

VI. A possible correlation of breast cancer cell proliferation and estrogen sulfotransferase activity

As was indicated in the preceding sections, SULT1E1 is present mainly in the normal breast cell and is active at nanomolar concentrations of E_2, consequently, it can block cell proliferation by the formation of the inactive E_2S. However, in breast cancer cells, SULT1A1 is present and active only at micromolar concentrations; most of the E_2 remains in an unconjugated form and can be involved in cell proliferation. The data suggest that there is an alteration of sulfotransferase quality and activity in the evolution of the breast cells from normal to cancerous. These effects are represented in Figures 9 and 10.

The progestins, promegestone, medrogestone and nomegestrol acetate, as well as tibolone and its metabolites, have an anti-proliferative effect on breast cancer cells (see section V). Consequently, we suggest a possible correlation of the stimulatory effect of sulfotransferase by these compounds which can decrease the levels of E_2 and consequently that of cell proliferation (Figure 11).

VII. Discussion

In the human breast, normal or cancerous, sulfotransferase activity is of vital importance as this enzyme can transform

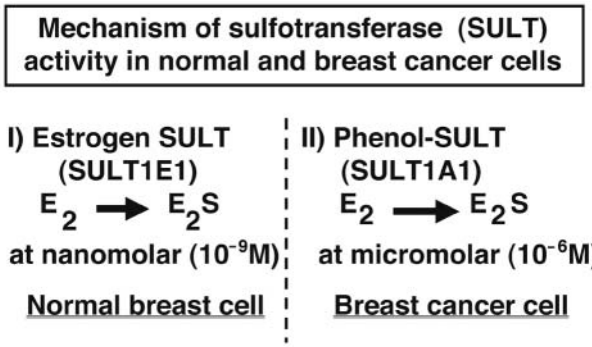

Figure 9. *Mechanism of sulfotransferase (SULT) activity in normal and breast cancer cells. In normal breast cells the sulfotransferase SULT1E1 is predominant and functions at physiological (nanomolar) concentrations of estradiol to form estradiol sulfate which is biologically inactive. This enzyme is absent from breast cancer cells, where the phenol-SULT (SULT1A1) activity acts only at micromolar (non-physiological) concentrations of estradiol.*

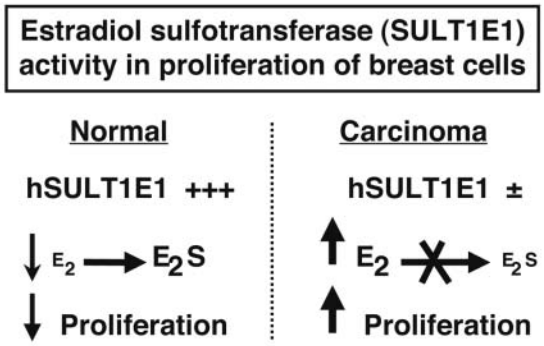

Figure 10. *Effects of estradiol sulfotransferase (SULT1E1) activity on the proliferation of breast cancer cells. In normal breast cells proliferation is inhibited, as estradiol sulfate (E_2S) formed by the SULT1E1 activity is biologically inactive. In contrast, in breast cancer cells SULT1E1 activity is very low or absent and E_2 can stimulate proliferation since E_2S is not formed.*

active estrogens (particularly estradiol) into biologically inactive sulfates. The very interesting observation of Falany and Falany (8) that in normal breast cells sulfotransferase acts at nanomolar concentrations and in breast cancer cells at micromolar concentrations indicates that there are qualitative and quantitative variations in the activity of this enzyme in the evolution of breast cells from normal to cancerous. The fact that various progestins (promegestone, medrogestone, nomegestrol acetate) and tibolone and its metabolites can stimulate estrogen sulfotransferase in breast cells at low concentrations indicates that the various compounds reported here could be involved as coactivators in the control of the sulfotransferase enzymes in the breast.

This stimulation of SULT1E1 can significantly decrease the levels of unconjugated estrogens (the active form) in breast cancer tissue and consequently block the proliferative effect of the hormone. As the different progestins tested are simultaneously antiproliferative agents against breast cancer cells and on DMBA tumors, a correlation can be hypothesized between the stimulatory effect of sulfotransferase and proliferation in the breast cells as indicated in Figures 9 to 11. The possible transformation of the sulfotransferases in agreement with the transformation of breast cells from normal to cancerous is a very attractive aspect of the quantitative bioformation of estrogen sulfates in breast tissues. However, the existence of possible co-factors (stimulatory or repressive) in the enzyme activity and its expression, as well as the local tissue concentration of estrogens in the breast, need to be explored. It is interesting to mention that these progestins and tibolone can also decrease sulfatase activity in breast cancer tissue. These dual effects exerted by the same compound: stimulation of sulfotransferase and inhibition of sulfatase activities,

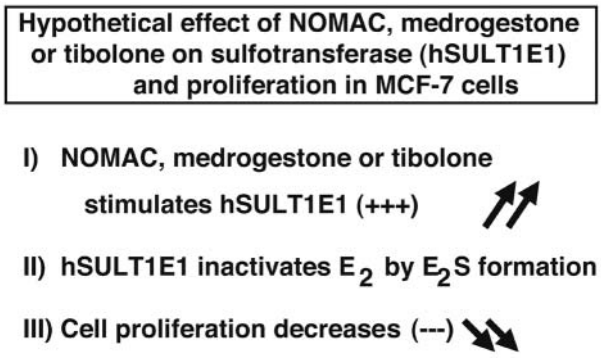

Figure 11. *Hypothetical effects of nomegestrol acetate, medrogestone or tibolone on human estrogen sulfotransferase (hSULT1E1) and proliferation in T-47D and MCF-7 breast cancer cells. Nomegestrol acetate, medrogestone or tibolone can stimulate hSULT1E1 in the cancer cell, hence the effect of estradiol is inactived, forming estradiol sulfate and consequently cell proliferation is inhibited.*

contribute to the displacement of the tissue concentration equilibrium between free and conjugated estrogens to favour the formation of the biologically inactive estrogen sulfates.

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