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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

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The evaluation of estrogens (estrone, estradiol, and their sulfates) in the breast tissue of post-menopausal patients with breast cancer indicates high levels, particularly of estrone sulfate (E_1S) which is 15–25 times higher than in the plasma. Breast cancer tissue contains the enzymes necessary for local synthesis of estradiol and it was demonstrated that, despite the presence of the sulfatase and its messenger in hormone-dependent and hormone-independent breast cancer cells, this enzyme operates particularly in hormone-dependent cells. Different progestins: Nomegestrol acetate, Promegestone, progesterone, as well as Danazol, can block the conversion of E_1S to E_2 very strongly in hormone-dependent breast cancer cells. The last step in the formation of estradiol is the conversion of E_1 to this estrogen by the action of 17β-hydroxysteroid dehydrogenase. This activity is preferentially in the reductive direction (formation of E_2) in hormone-dependent cells, but oxidative ($E_2 \rightarrow E_1$) in hormone-independent cells. Using intact hormone-dependent cells it was observed that Nomegestrol acetate can block the conversion of E_1 to E_2. It is concluded, firstly, that in addition to ER mutants other factors are involved in the transformation of hormone-dependent breast cancer to hormone-independent, this concerns the enzymatic activity in the formation of E_2; it is suggested that stimulatory or repressive factor(s) involved in the enzyme activity are implicated as the cancer evolves to hormone-independence; secondly, different drugs can block the conversion of E_1S to E_2. Clinical trials of these "anti-enzyme" substances in breast cancer patients could be the next step to investigate new therapeutic possibilities for this disease.

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INTRODUCTION

Recent statistical information indicates that in the United States, one woman in 8 will be at risk of having breast cancer during her life; the values are one in 12 for countries of the European Community and one in 80 for Japan. The yearly (1993) number of new cases is indicated in Table 1. It is notable that the greater percentage of deaths is in Great Britain. The incidence of breast cancer represents 30% of all cancer in women and 20% of deaths due to this disease.

Two thirds of breast cancers are manifested during the post-menopausal period and the great majority (about 95%) are initially hormone-dependent, where

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Table 1. Incidence and mortality of breast cancer

Country	New cases/year (1993)	Mortality	%
U.S.A.	200,000	50,000	25
France	45,000	12,000	27
Great Britain	30,000	17,000	57

estradiol (E_2) plays a capital role in their development and evolution [1–4]. After a period that can last for several years, the tumor becomes hormone-independent. The mechanism of this conversion is not yet established, but recent information provides a better understanding and suggests hypothetical mechanisms of the transformation by which the cancer cells become hormone-independent. The discovery of estrogen receptor (ER) mutants [5–7] can be one explanation of this conversion. In hormone-dependent cells, the association 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 [8–11], the ER becomes "non-functional" and despite estrogen binding, the cell fails to respond to hormone action.

The fact that ERs are present in the cancer cells but are "non-functional" can explain that 35–40% of patients with ER-positive tumors fail to respond to the therapy with anti-estrogens [12, 13].

The conversion of the cancer cell from hormone-dependent to hormone-independent is a very complex mechanism which can involve stimulatory and inhibitory growth factors, oncogenes, but recent data indicated significant differences in these types of cell concerning the activities of two enzymes: the sulfatase and the 17β-hydroxysteroid dehydrogenase (17β-HSD) [14, 15], which are implicated in E_2 biosynthesis in the tissue itself. This paper includes recent information concerning sulfatase and 17β-HSD activities in hormone-dependent and hormone-independent breast cancer cells, as well as their control by various drugs.

ESTROGEN CONCENTRATIONS IN BREAST CANCER PATIENTS

Estrone sulfate is quantitatively the most important circulating estrogen (E_1S) in the menstrual cycle and in post-menopausal women [16, 17]. As during the post-menopausal period two thirds of breast cancers are manifested and in that period, as a consequence of the ovary being "non-functional", the circulating estrogens are very low, it was interesting to evaluate the tissular levels of the various estrogens in post-menopausal patients with breast cancer.

Figure 1 gives the concentration values of E_1 and E_2 and Fig. 2 those of E_1S and estradiol sulfate (E_2S) in the breast tissues and plasma of pre- and post-menopausal patients with breast cancer. The high estrogen concentrations in the tumor of post-menopausal

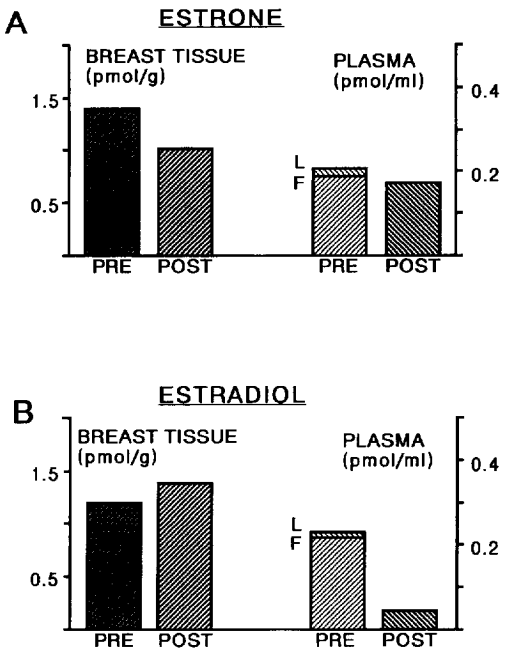

Fig. 1. Concentrations of estrone (A) and estradiol (B) in the breast tissue and plasma of pre- and post-menopausal patients with breast cancer. The concentrations of the two estrogens were evaluated in the plasma and in the breast tumors of pre- and post-menopausal patients by radioimmunoassay (RIA). Values are expressed as the mean of 10 determinations.

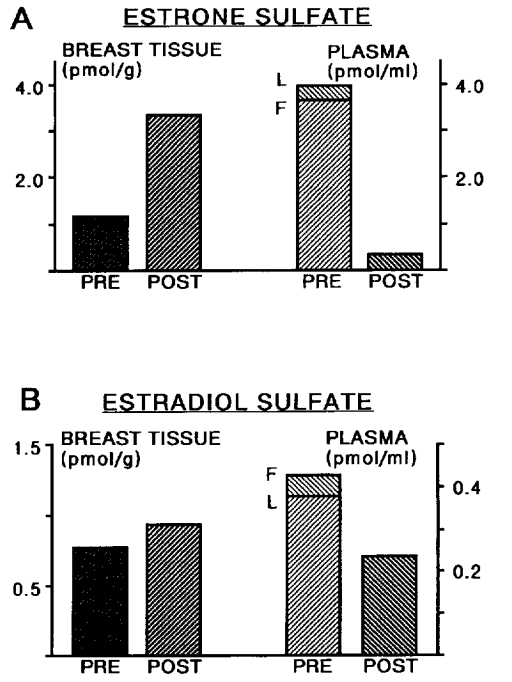

Fig. 2. Concentrations of E_1S (A) and of E_2S (B) in the breast tissue and plasma of pre- and post-menopausal patients with breast cancer. The concentrations of the two estrogen sulfates were evaluated in the plasma and in the breast tumors of pre- and post-menopausal patients by radioimmunoassay (RIA). E_1 and E_2 were evaluated by RIA after solvolysis of the respective estrogen sulfates. Values are expressed as the mean of 10 determinations.

Table 2. Ratio concentration in the tumor tissue and plasma of estrone (E_1), estradiol (E_2) and their sulfates (E_1S, E_2S) in human breast cancer

Patients	E_1	E_2	E_1S	E_2S
Pre-menopausal	7	5	0.3	2
Post-menopausal	6	22	9.0	4

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 values obtained with 10–15 patients.

patients are noteworthy, in particular that of E_1S. This intriguing data could be explained by the fact that the breast tumor contains all the enzymatic systems for the formation of unconjugated [18–24] or sulfoconjugated estrogens [25–27]. Another explanation of this tissular accumulation of estrogens could be the presence of proteins or other substances which can specifically bind the estrogens. Table 2 gives the tumor:plasma concentration ratios of the various estrogens. As observed, the gradient of tumoral tissues to blood increases very significantly for E_2 and E_1S when pre- and post-menopausal patients are compared.

SULFATASE ACTIVITY IN HORMONE-DEPENDENT AND HORMONE-INDEPENDENT HUMAN BREAST CANCER CELLS AND EFFECTS OF VARIOUS DRUGS

In previous studies in this laboratory, it was observed that using hormone-dependent breast cancer cells (e.g. MCF-7, T-47D), E_1S in physiological concentrations (5×10^{-9} M), is converted, in a great proportion, to E_2; however, very little or no conversion was obtained with the hormone-independent cells (e.g. MDA-MB-231, MDA-MB-436, MDA-MB-468) [24, 28, 29]. As an example, after incubation of [^{3}H]E_1S (5×10^{-9} M) with the T-47D cells the intracellular concentration of E_2 was 358–1912 pg/mg DNA, with the MCF-7 cells it was 84–280 pg/mg DNA; however, the values of E_2 were only 4–10 pg/mg DNA after incubation of E_1S with the various hormone-independent cells indicated above.

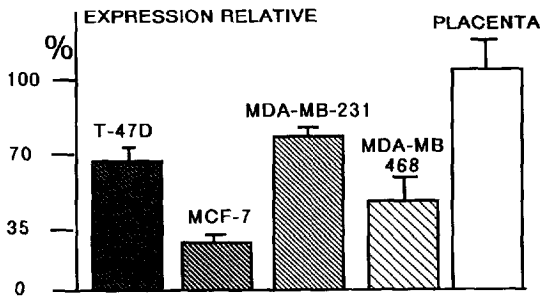

Fig. 3. Relative expression of mRNA sulfatase in various human mammary cancer cell lines using reverse transcription-polymerase chain reaction amplification. The values were calculated using a densitometer (ULTROSCAN LKB XL) and the value of 100 was assigned to the expression of the sulfatase mRNA of the human term placenta. [quoted from Ref. 30].

In another series of studies, the estrone sulfate-sulfatase activity was evaluated in the homogenates of these different cells and the surprise was that in the hormone-independent cells the activity of this enzyme was relatively high (e.g. in the MDA-MB-231 cells, the V_{max} is 5–10 times higher than in the MCF-7 cells) [14, 30] (Table 3).

In order to explore the mechanism of the sulfatase activity, we studied the expression of this enzyme in different breast cancer cells. Using reverse transcriptase polymerase chain reaction amplification we observed that the mRNA of this enzyme is present in the hormone-dependent and hormone-independent cells, and there was a correlation between enzyme activity and expression of the mRNA of this enzyme [30] (Fig. 3 and Table 3). With this data it is concluded that the factor(s) involved in sulfatase action (enzyme and its mRNA) are present in both hormone-dependent and -independent breast cancer cells, but only operate in the former.

Table 3. Estrone sulfatase (STS) activity in the hormone-dependent (MCF-7, T-47D) and hormone-independent (MDA-MB-231, MDA-MB-468) human mammary breast cancer cells and in human term placenta, and relative STS mRNA expression

	V_{max} (nmol E_1/mg protein/h)	Relative STS mRNA expression (%)
T-47D	52.7 ± 7.6	65
MCF-7	5.2 ± 2.3	20
MDA-MB-231	19.3 ± 6.3	76
MDA-MB-468	8.4 ± 1.7	45
Placenta	78.4 ± 11.6	100

Values are the means ± SEM of duplicate determinations of 3–5 experiments [quoted from Ref. 30].

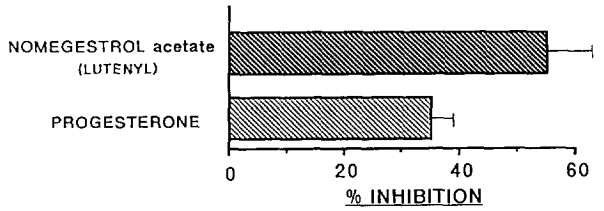

Fig. 4. Inhibitory effect of various progestins on the conversion of E_1S to E_2 in T-47D mammary cancer cells. The percentage of inhibition was obtained by calculating the ratio (control − test)/control × 100. The T-47D cells were incubated with 5×10^{-9} M [^{3}H]E_1S and its conversion to E_2 (1050 ± 260 pg/mg DNA) was assigned 100 (control). Cells were treated with Nomegestrol acetate or progesterone (5×10^{-6} M). The data represent the average of 4–6 determinations.

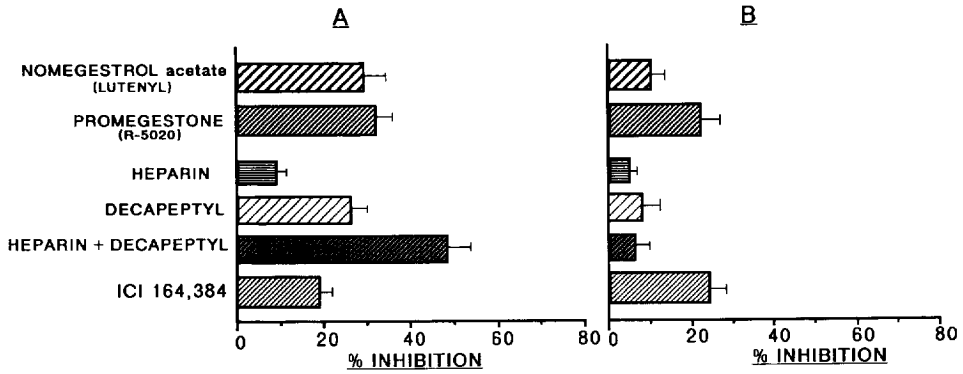

Fig. 5. Comparative inhibitory effects of various compounds on the sulfatase activity in the homogenates of hormone-dependent MCF-7 (A) and hormone-independent MDA-MB-231 (B) breast cancer cells. Sulfatase assays were performed on total cell homogenates of the estrogen-dependent MCF-7 and the estrogen-independent MDA-MB-231 breast cancer cells. The concentrations of the different compounds were: E_1S, 2×10^{-6} M; heparin, 10 μg/ml; other substances, 10^{-6} M. Control values (E_1S alone) correspond to 485 ± 57 and 2200 ± 194 pmol/mg protein/h for MCF-7 and MDA-MB-231 cells, respectively. Percentage of inhibition was obtained by calculating the ratio: (control − test)/control × 100. Data are the mean ± SEM of duplicate determinations of 4–6 experiments.

As E_1S is found in relatively high concentrations in breast cancer tissues, particularly in post-menopausal patients [29, 31] (see Fig. 2) and the sulfatase activity is also higher [21–23, 29–31], in the last few years it was of particular interest to explore different substances that can act as anti-sulfatase agents.

Figure 4 gives a comparative effect on the transformation of E_1S to E_2 using Nomegestrol acetate (Lutenyl), and progesterone itself, after 24 h incubation of E_1S (5×10^{-9} M) with the T-47D hormone-dependent cells. It is observed that these different drugs provoke a significant decrease in the intracellular concentration of E_2, and the effect is particularly intense with Nomegestrol acetate. In order to explore a "direct effect" on the enzyme, Nomegestrol acetate, R-5020, the GnRH agonist analog Decapeptyl, in the presence or absence of heparin, as well as the anti-estrogen ICI 164,384, were tested for the effect on the sulfatase activity of a hormone-dependent (MCF-7) and a hormone-independent (MDA-MB-231) cell line (Fig. 5). In the MCF-7 cells, a significant inhibitory effect of this enzyme was obtained with Nomegestrol acetate, R-5020 and Decapeptyl in the presence of heparin, however, the effect of these substances is very limited in hormone-independent cells (e.g MDA-MB-231).

17β-HYDROXYSTEROID DEHYDROGENASE (17β-HSD) ACTIVITY IN HORMONE-DEPENDENT AND HORMONE-INDEPENDENT BREAST CANCER CELLS

The final step for the conversion of E_1 to E_2 is obtained by the action of a 17β-HSD; however, this enzyme can operate in both directions: the reductive ($E_1 \rightarrow E_2$) or the oxidative ($E_2 \rightarrow E_1$). When a physiological concentration (5×10^{-9} M) of E_1 was incubated with the intact hormone-dependent MCF-7 or T-47D cells for 24 h, 80–90% was converted to E_2, whereas when E_2 was incubated in the same experimental conditions, 90% remained unchanged. However, when a physiological concentration (5×10^{-9} M) of E_2 was incubated with the intact hormone-independent MDA-MB-231, MDA-MB-436, or Hs-578 cells for 24 h, 70–85% was converted to E_1, and when E_1 was incubated in the same experimental conditions, 85% remained unchanged [15].

The data show very clearly that the reductive ($E_1 \rightarrow E_2$) pathway is preferential in hormone-dependent breast cancer cells, while in hormone-independent cells the 17β-HSD activity is orientated to the oxidative direction.

In order to explore the effects of various substances on the $E_1 \rightarrow E_2$ transformation, the hormone-dependent T-47D cells were incubated with E_1 (5×10^{-9} M) in the absence or presence of Nomegestrol acetate, R-5020,

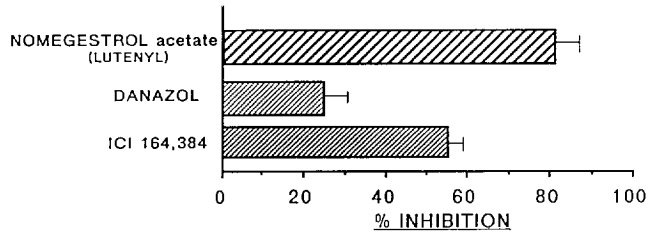

Fig. 6. Inhibitory effects of various drugs on the reductive 17β-HSD activity of estrone ($E_1 \rightarrow E_2$) in intact T-47D breast cancer cells. [^{3}H]E_1 at 5×10^{-9} M was incubated with the T-47D cells for 24 h at 37°C in the absence (control) or presence of 5×10^{-6} M of Nomegestrol acetate, Danazol, or ICI 164,384. The calculation of E_2 was carried out after isolation of the hormone by thin layer chromatography. Control values were 5.8 ± 1.3 pmol E_2/mg DNA. The percentage of inhibition was obtained by calculating the ratio: (control − test)/control × 100. Data are the mean ± SEM of 3–4 experiments.

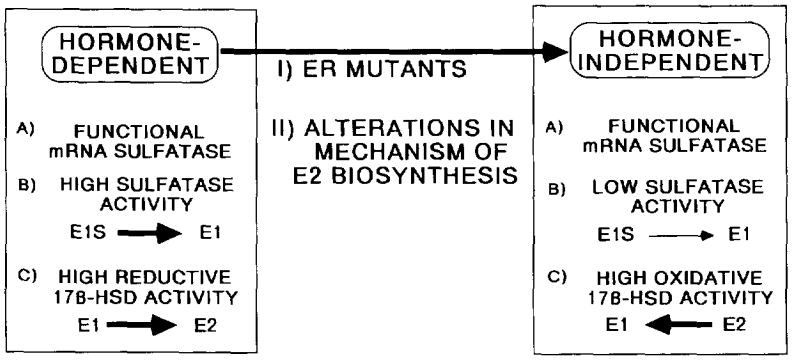

Fig. 7. Hypothetical concept of the factors involved in the transformation of breast cancer from hormone-dependent to hormone-independent. The transformation of breast cancer cells from hormone-dependent to hormone-independent is a very complex mechanism in which only limited information is available at present. One interesting discovery is the presence of ER mutants, the ER evolve to "non-functional" and the hormone becomes inoperative. Another attractive aspect concerns the enzymes involved in E_2 formation. The sulfatases are very active for the intact hormone-dependent (HD) cells, but in hormone-independent (HI) cells, in spite of the fact that the enzyme and its mRNA are present, the activity is very low, suggesting the presence of activator factor(s) in HD or repressive factor(s) in HI. Similarly, in the interconversion of $E_1 \leftrightarrow E_2$, the factors for the formation of E_2 (the biological form) are very active in HD, whereas in HI the factor(s) act to the formation of the less active estrogen E_1.

Danazol, and the anti-estrogen ICI 164,384 at a concentration of 10^{-6} M. As indicated in Fig. 6, Nomegestrol acetate was very active in blocking the conversion of E_1 to E_2; a similar effect was observed with the anti-estrogen ICI 164,384, whereas no significant effect was obtained with Danazol.

CONCLUSIONS AND PERSPECTIVES

Recent findings in this laboratory and others demonstrate very clearly that human breast cancer tissue contains the enzymes necessary for the formation of E_2: sulfatase, aromatase, 17β-HSD. Sulfotransferases, which transform estrogens into their sulfates are also present in this tissue. Evaluation of various estrogens: E_1, E_2, and their sulfates, in cancer tissues (Figs 1 and 2) of post-menopausal patients shows that the concentrations of these estrogens are relatively high and many times those found in the plasma. This accumulation of estrogens in the carcinoma tissue at a period in which the ovary is "non-functional" is very intriguing and complementary information is required for clarification and better understanding.

Comparative data show that in breast cancer tissues, the sulfatase activity is 10–100 times higher than the aromatase, suggesting that the main pathway for the formation of E_2 in this tissue is through the hydrolysis of E_1S.

In another series of studies it was well established that mRNA of sulfatase is present in different breast cancer cells and that the expression of this messenger is correlated with sulfatase activity; however, the capacity to hydrolyze E_1S in some cell lines does not reflect the levels of sulfatase activity, for instance: for the hormone-independent MDA-MB-231 cells the sulfatase activity is very low in the intact cells, but becomes very active after homogenization of the cells. The data indicate that the presence of the enzyme and its mRNA are not sufficient, suggesting that other "factor(s)" are involved in the mechanism for the hydrolysis of E_1S; these "factor(s)" could be absent from hormone-independent cells.

Another example in the alteration of the enzyme activities implicated in the formation of E_2 as the breast cancer evolves from hormone-dependent to hormone-independent concerns 17β-HSD. It was observed that in the hormone-dependent cells the activity of the 17β-HSD is to the formation of E_2, whereas in the hormone-independent cells the tendency is to the oxidative form (formation of E_1). All this information, and the interesting finding of ER mutants, suggests that the evolution of cancer to hormone-independence is a very complex mechanism. A present hypothetical concept of this transformation is represented in Fig. 7.

In the exploration of "sulfatase inhibitors" using isolated breast cancer cells, interesting findings were obtained with the progestins: Nomegestrol acetate and Promegestrone (R-5020). Concerning 17β-HSD activity, the significant effect of Nomegestrol acetate in blocking the conversion of E_1 to E_2 in the T-47D hormone-dependent cells is notable.

In conclusion, it is well established at present that in isolated models (intact breast cancer cells) various drugs, including progestins, anti-estrogens and Danazol, can inhibit the enzymes involved in the transformation of E_1S to E_2. Clinical trials of these "anti-enzyme" drugs in breast cancer patients could be the next step to investigate new therapeutic possibilities for this disease.

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