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Control of sulfatase and sulfotransferase activities by medrogestone in the hormone-dependent MCF-7 and T-47D human breast cancer cell lines

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Abstract

In the present study, we explored the effect of the progestin medrogestone on the sulfatase and sulfotransferase activities in the hormone-dependent MCF-7 and T-47D human breast cancer cell lines. After 24 h incubation at 37°C of physiological concentrations of estrone sulfate ([^{3}H]-E_1S: 5×10^{-9} mol/l), it was observed that this estrogen was converted in a great proportion to E_2 in both cell lines. Medrogestone significantly inhibits this transformation, at all the concentrations tested (5×10^{-8} to 5×10^{-5} mol/l), in both cell lines. The IC_{50} values were 1.93 μmol/l and 0.21 μmol/l in MCF-7 and T-47D cells, respectively. In another series of studies, after 24 h incubation at 37°C of physiological concentrations of estrone ([^{3}H]-E_1: 5×10^{-9} mol/l), the sulfotransferase activity was detectable in both cell lines. Estrogen sulfates (ES) are found exclusively in the culture medium, which suggests that as soon as they are formed they are excreted into the medium. Medrogestone has a biphasic effect on sulfotransferase activity in both cell lines. At low doses: 5×10^{-8} and 5×10^{-7} mol/l, this compound stimulates the enzyme by +73.5 and 52.7%, respectively, in MCF-7, and by 84.5 and 62.6% in T-47D cells. At high concentrations: 5×10^{-6} and 5×10^{-5} mol/l, medrogestone has no effect on MCF-7 cells, but inhibits the sulfotransferase activity in T-47D cells by −31.4% at 5×10^{-5} mol/l.

In conclusion, the inhibitory effect provoked by medrogestone on the enzyme involved in the biosynthesis of E_2 (sulfatase pathway) in estrogen-dependent breast cancer, as well as the stimulatory effect on the formation of the inactive ES, support a probable anti-proliferative effect of this progestin in breast tissue. Clinical applications of these findings can open new therapeutic possibilities for this disease.

Keywords: Breast cancer; MCF-7 and T-47D cell lines; Sulfatase; Sulfotransferase; Progestins; Medrogestone

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 her 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 estradiol (E_2) is an important risk factor for the genesis and evolution of breast tumor, and that most (about 95%) in their early stage are estrogen-sensitive [1–3]. Two thirds of breast cancers occur during the post-menopausal period when the ovaries have ceased to be functional and, despite the low levels of circulating estrogens, the tissular concentrations of estrone (E_1), E_2 and their sulfates, are

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several times higher than those found in blood and in normal breast tissue, suggesting a tumoral accumulation of these hormones [5–9].

There is substantial information that mammary cancer tissue contains all the enzymes necessary for the local biosynthesis of E_2 from circulating precursors [10]. Two principal pathways are implicated in the last steps of E_2 formation in breast cancer tissues: the 'pathway' which transforms androgens into estrogens [11–13], and the 'sulfatase pathway' (estrone-sulfatase, E.C.: 3.1.6.2.) which converts estrone sulfate (E_1S) into E_1 [14–18] which is then transformed into E_2 by the reductive 17β-hydroxysteroid dehydrogenase (17β-HSD) activity [19–21]. Quantitative evaluation indicates that in human breast tumor E_1S 'via sulfatase' is a much more likely precursor for E_2 than are androgens 'via aromatase' [4,6,22,23].

Steroid sulfotransferases (ST), which convert estrogens into their sulfates (ES), are also present in breast cancer [17,24–26]. Sulfotransferase activity is important, firstly 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, secondly, because sulfoconjugates are biologically inactive and do not bind to the estrogen receptor (ER). We have previously shown that some progestins (e.g. promegestone) can inhibit sulfatase activity and its mRNA, and regulate EST activity and its mRNA, in a dose-dependent manner in both MCF-7 and T-47D breast cancer cells [27,28].

In the present studies, we tested the capacity of medrogestone (Prothil®), a synthetic pregnane derivative which has the same physiological activities as progesterone itself, to control the level of ES in hormone-dependent MCF-7 and T-47D human breast cancer cell lines, by modulating the sulfatase and EST activities.

2. Materials and methods

2.1. Chemicals

[6,7–^{3}H(*N*)]-Estrone (^{3}H-E_1) (49 Ci/mmol), [6,7–^{3}H(N)]-estrone-3-sulfate (^{3}H-E_1S), ammonium salt (53 Ci/mmol), [4–^{14}C]-estradiol (^{14}C-E_2) (57 mCi/mmol) and [4–^{14}C]-estrone (4-^{14}C-E_1) (51 mCi/mmol) were purchased from New England Nuclear Division (DuPont de Nemours, Les Ulis, France). The purity of the radioisotopes was assessed by thin-layer chromatography (TLC) in the appropriate system before use. Unlabeled E_1S, E_1 and E_2 were obtained from Sigma-Aldrich Chimie, (St Quentin Fallavier, France). Medrogestone (6,17-dimethyl pregna-4,6-diene-3,20-dione) was a gift from Solvay Arzneimittel (Hannover, Germany). The structure of Medrogestone is given in Fig. 1. All chemicals were of the highest purity available.

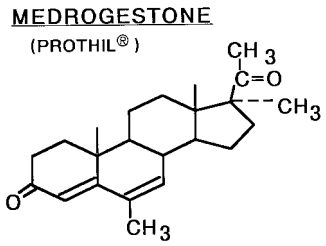

Fig. 1. Structure of the progestin Medrogestone (Prothil®).

2.2. Cell culture

The hormone-dependent MCF-7 and T-47D human mammary cancer cell lines were kindly provided by Dr S.A.W. Fuqua (Texas University, San Antonio, USA). The cells were routinely grown in Eagle's Minimal Essential Medium (MEM) buffered with 10 mmol/l HEPES (pH 7.6), supplemented with 2 mmol/l L-glutamine, 100 U/ml penicillin-streptomycin (A.T.G.C., Noisy-Le-Grand, France) and 5% fetal calf serum (FCS) (D.A.P., Vogelgrun, France) for T-47D cells, or 10% FCS for MCF-7 cells, and incubated at 37°C in a humidified atmosphere of 5% CO_2. Media were changed twice a week. The cells were passed every 10–12 days and replated in 75 cm^2 flasks (A.T.G.C.) at 3×10^6 cells/flask for MCF-7 and T-47D cells. Four days before the experiments, the cells were transferred to MEM containing 5% steroid-depleted treated FCS which had been treated overnight at 4°C with dextran-coated charcoal (DCC) (0.1–1% w/v, DCC-FCS) to remove endogenous steroids.

2.3. Isolation and quantification of [^{3}H]-estradiol from human mammary cancer cells incubated with [^{3}H]-estrone sulfate

Cells near confluence were cultivated in MEM-DCC-FCS (10 ml) with the addition of 5×10^{-9} mol/l of [^{3}H]-E_1S alone or in the presence of medrogestone, prepared in ethanol (final concentration $<0.5\%$) at the concentration range of 5×10^{-5} to 5×10^{-8} mol/l. Control cells received ethanol vehicle only. Twenty-four hours later, the medium was removed, the cells washed twice with ice-cold Hank's balanced salt solution (HBSS, calcium–magnesium free) and harvested with 15 ml HBSS by scraping with a rubber policeman. After centrifugation, the pellet was precipitated by 80% ethanol and the radioactivity extracted for at least 24 h at −20°C. The cellular radioactivity uptake was determined in the ethanolic supernatant, and the DNA content in the remaining pellet was evaluated

according to Burton [29]. After evaporation of the organic phase, the two extracts were redissolved in 50 μl of ethanol and the qualitative analysis and quantitative evaluation of E_2 were carried out after isolation by TLC on silica gel $60F_{254}$ plates (Merck, Darmstadt, Germany) developed with chloroform–ethylacetate (4:1, v/v). [^{14}C]-E_2 (5000 dpm) was added to monitor analytical losses and unlabeled E_1S, E_1 and E_2 (50 μg) were used as carriers and reference indicators. After visualization of the estrogens under UV at 254 nm, the appropriate areas were cut off into small pieces, placed in liquid scintillation vials with ethanol (0.5 ml) and allowed to extract for 30 min. Three ml of Opti-fluor (Packard, Rungis, France) were added and the vials analyzed for ^{3}H and ^{14}C contents with quench correction by external standardization. The quantitative evaluation of the transformation of [^{3}H]-E_1S to [^{3}H]-E_2, corresponding to the sulfatase activity at 24 h, was calculated as a percentage of the total radioactivity associated with the cells and then expressed as pmol/mg E_2 formed/mg DNA.

2.4. Isolation and quantification of [^{3}H]-estrogen sulfates from human mammary cancer cells incubated with [^{3}H]-estrone

Experiments were performed as described above, with the exception that cells were incubated with [^{3}H]-E_1. The sulfoconjugated estrogens present in the ethanolic extracts of the cell compartment and culture medium were quantified after isolation by TLC on silica gel $60F_{254}$ plates developed using the mixture of ethylacetate/methanol/ammonium hydroxide (75:25:2 v/v) for the separation of steroid sulfates. [^{14}C]-E_1 (5000 dpm) was added to monitor analytical losses and unlabeled estrogens E_1, E_2 E_1S or E_2S, (50 μg of each) were used as carriers and reference standards. After visualization of the estrogens under UV, the appropriate areas were cut off into small pieces, placed in liquid scintillation vials with ethanol (0.5 ml) and allowed to extract for 30 min. Three ml of Opti-fluor were added and the vials analyzed for ^{3}H and ^{14}C contents. The quantitative evaluation was done by calculating the percentage of the total radioactivity associated with the cells or the medium and then expressed as pmol/mg ES formed/mg DNA.

2.5. Statistical analysis

Data are expressed as the mean ± standard error of the mean (S.E.M.) values. Students t -test was used to assess the significance of the differences between means; p-values ≤ 0.05 were considered significant.

Table 1
Dose–response effect of medrogestone (Prothil®) on the conversion of estrone sulfate (E_1S) To estradiol in hormone-dependent MCF-7 and T-47D human breast cancer cell lines. $^*p < 0.05$ versus control value (non-treated cells; [^{3}H]-E_1S alone). $^{**}p < 0.01$ versus control value (non-treated cells; [^{3}H]-E_1S alone)[a]

	Estradiol (in the cells, pmol/mg DNA)	
	MCF-7 cells	T-47D cells
[^{3}H]-E_1S alone	0.97 ± 0.07	2.65 ± 0.19
+ Medrogestone		
5×10^{-8} mol/l	$0.76 \pm 0.05^*$	$1.62 \pm 0.11^*$
5×10^{-7} mol/l	$0.55 \pm 0.05^*$	$1.24 \pm 0.10^*$
5×10^{-6} mol/l	$0.41 \pm 0.04^*$	$0.59 \pm 0.07^{**}$
5×10^{-5} mol/l	$0.15 \pm 0.02^{**}$	$0.26 \pm 0.04^{**}$

[a] [^{3}H]-E_1S: 5×10^{-9} mol/l, was incubated with the MCF-7 or T-47D cells for 24 h at 37°C in the absence or presence of medrogestone in the range of 5×10^{-8}–5×10^{-5} mol/l. The calculation of estradiol (in the cell compartment) was carried out after isolation of the hormones as indicated in Materials and Methods. The data represent the average ± SEM of 3–4 duplicate independent experiments.

3. Results

3.1. Effect of medrogestone on the conversion of estrone sulfate (E_1S) to estradiol (E_2) in hormone-dependent MCF-7 and T-47D human breast cancer cell lines

As the sulfatase pathway is the main route for the intracellular biosynthesis of E_2 in breast cancer cells, it was of particular interest to test the effect of the progestin, medrogestone, on this conversion. The hormone-dependent MCF-7 and T-47D breast cancer cell

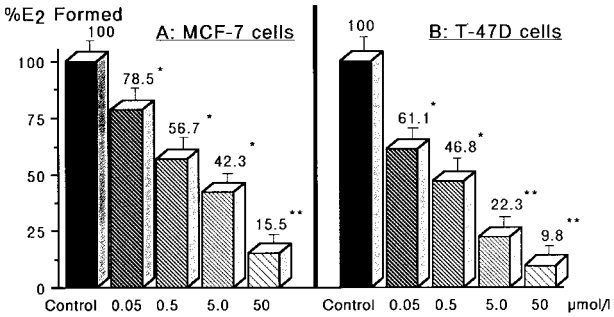

Fig. 2. Effects of Medrogestone (Prothil®) on the conversion of estrone sulfate (E_1S) to estradiol (E_2) in the hormone-dependent MCF-7 and T-47D human breast cancer cell lines. Preconfluent cells were incubated for 24 h at 37°C with a physiological concentration of estrone sulfate ([^{3}H]-E_1S: 5×10^{-9} mol/l) alone (control: non-treated cells) or in the presence of Medrogestone at a concentration range from 0.05 to 50 μM. Qualitative and quantitative analyses of E_2 in the cell compartment were performed by thin-layer chromatography as indicated in Materials and Methods. Results (pmol of E_2 formed/mg DNA) are expressed in percent (%) of control values considered as 100%. The data are the mean ± S.E.M. of duplicate determinations of 3–4 independent experiments. $^*p < 0.05$ versus control value (non-treated cells). $^{**}p < 0.01$ versus control value (non-treated cells).

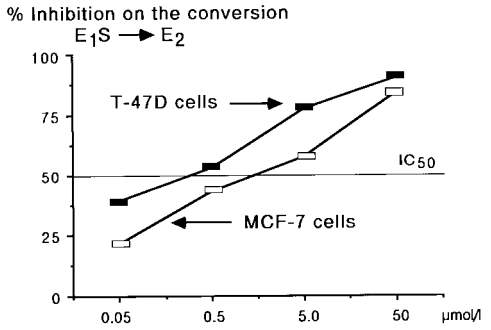

Fig. 3. Comparative inhibitory effects of Medrogestone (Prothil®) on the conversion of estrone sulfate (E_1S) to estradiol (E_2) in the hormone-dependent MCF-7 and T-47D human breast cancer cell lines. Preconfluent cells were incubated for 24 h at 37°C with a physiological concentration of estrone sulfate ([^{3}H]-E_1S: 5×10^{-9} mol/l) alone (control: non-treated cells) or in the presence of Medrogestone at a concentration range of 0.05 to 50 µM. The percentage of the inhibitory effect (in pmol E_2 formed/mg DNA) was obtained by calculating the ratio: [(control–test)/control] × 100. The data are the mean ± S.E.M. of duplicate determinations of 3–4 independent experiments. The IC_{50} values correspond to the concentration of Medrogestone needed to obtain 50% inhibition vs. control ([^{3}H]-E_1S alone). The values were determined by using non-linear regression analyses.

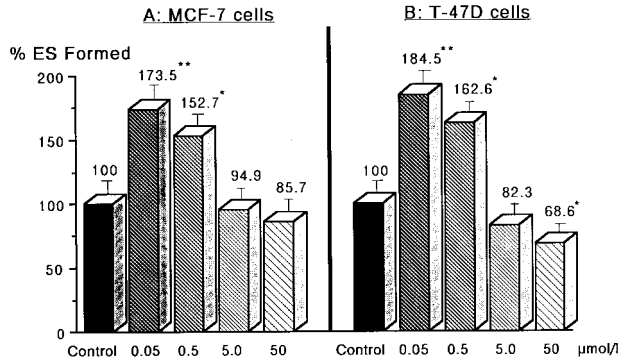

Fig. 4. Effects of Medrogestone (Prothil®) on the conversion of estrone (E_1) to estrogen sulfates (ES) in the hormone-dependent MCF-7 and T-47D human breast cancer cell lines. Preconfluent cells were incubated for 24 h at 37°C with a physiological concentration of estrone ([^{3}H]-E_1: 5×10^{-9} mol/l) alone (control: non-treated cells) or in the presence of Medrogestone at a concentration range of 0.05–50 µM. Qualitative and quantitative analyses of ES in the culture medium were performed by thin-layer chromatography as indicated in Materials and Methods. Results (pmol of ES formed/mg DNA) are expressed in percent (%) of control values considered as 100%. The data are the mean ± S.E.M. of duplicate determinations of three independent experiments. *$p < 0.05$ versus control value (non-treated cells). **$p < 0.01$ versus control value (non-treated cells).

cultures both have the capacity to transform physiological concentrations (5×10^{-9} mol/l) of [^{3}H]-E_1S, incubated for 24 h at 37°C, to E_2. The corresponding values of E_2 formed in the cell compartment indicate that the sulfatase activity is 2.7 times higher in T-47D cells (Table 1). Medrogestone has a significant dose-dependent inhibitory effect on the sulfatase pathway in MCF-7 and T-47D breast cancer cell lines at all the concentrations tested (5×10^{-8} to 5×10^{-5} mol/l) (Table 1 and Fig. 2). The effect is significantly more intense (~9 times) in the enriched-PR T-47D than in the MCF-7 cells, as shown by the IC_{50} values (corresponding to the concentrations of medrogestone needed to inhibit the formation of E_2 by 50%): 0.21 µmol/l and 1.93 µmol/l, respectively (Fig. 3).

Table 2
Dose–response effect of medrogestone (Prothil® on the conversion of estrone (E_1) to estrogen sulfates in hormone-dependent MCF-7 and T-47D human breast cancer cell lines. *$p < 0.01$ versus control value (non-treated cells; [^{3}H]-E_1 alone). **$p < 0.05$ versus control value (non-treated cells; [^{3}H]-E_1 alone)[a]

	Estrogen sulfates (in the medium, pmol/mg DNA)	
	MCF-7 cells	T-47D cells
[^{3}H]-E_1 alone	8.75 ± 0.7	12.94 ± 0.6
+ Medrogestone		
5×10^{-8} mol/l	15.18 ± 1.0*	23.87 ± 0.7*
5×10^{-7} mol/l	13.36 ± 0.6**	21.04 ± 0.8**
5×10^{-6} mol/l	8.30 ± 0.4	10.65 ± 0.4**
5×10^{-5} mol/l	7.50 ± 0.5	8.88 ± 0.5**

[a] [^{3}H]-E_1: 5×10^{-9} mol/l, was incubated with the MCF-7 or T-47D cells for 24 h at 37°C in the absence or presence of medrogestone in the range of 5×10^{-8}–5×10^{-5} mol/l. The calculation of estrogen sulfates (in the culture medium) was carried out after isolation of the hormones as indicated in Materials and Methods. The data represent the average ± S.E.M. of three duplicate independent experiments.

3.2. Effect of medrogestone on the conversion of estrone (E_1) to estrogen sulfates (ES) in hormone-dependent MCF-7 and T-47D human breast cancer cell lines

As sulfoconjugates are not biologically active, the formation of this conjugate in breast cancer cells can represent an important transformation pathway in the control of E_2. Consequently, it was interesting to explore if medrogestone can modulate estrogen sulfotransferase activity in MCF-7 and T-47D cells. After incubation of physiological concentrations (5×10^{-9} mol/l) of [^{3}H]-E_1 during 24 h at 37°C, estrogen sulfates are only detectable in the culture medium of MCF-7 and T-47D cells, the concentrations of ES formed were 8.75 and 12.94 pmol/mg DNA, respectively (Table 2). As no cofactor 3′-phosphoadenosine 5′-phosphosulfate was added to the cell cultures, the specific metabolism of each type of cells was maintained. Medrogestone has a bi-phasic effect on the sulfotransferase activity. At low concentrations (5×10^{-8} to 5×10^{-7} mol/l) medrogestone stimulates the formation of ES in both cells: +73.5 and +52.7% respectively for MCF-7 and 84.5 and 62.6% for T-47D cells, whereas at high concentrations (5×10^{-6}–5×10^{-5} mol/l) the sulfotransferase activity is not modified in MCF-7 cells and is

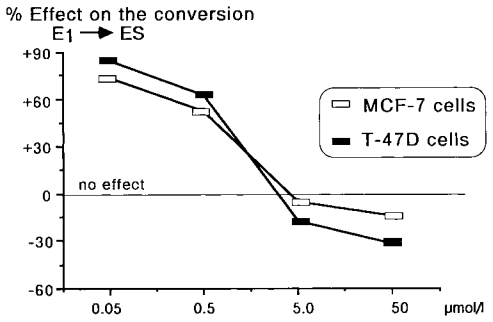

Fig. 5. Comparative effects of Medrogestone (Prothil®) on the conversion of estrone (E_1) to estrogen sulfates (ES) in the hormone-dependent MCF-7 and T-47D human breast cancer cell lines. Preconfluent cells were incubated for 24 h at 37°C with a physiological concentration of estrone ([^{3}H]-E_1: 5×10^{-9} mol/l) alone (control: non-treated cells) or in the presence of Medrogestone at a concentration range of 0.05–50 μM. The percentage of the effect (in pmol ES formed/mg DNA) was obtained by calculating the ratio: [(control–test)/control] × 100. The data are the mean ± S.E.M. of duplicate determinations of three independent experiments. The IC_{50} values correspond to the concentration of Medrogestone needed to obtain 50% inhibition vs. control ([^{3}H]-E_1 alone). The values were determined by using non-linear regression analyses.

inhibited by 31.5% in T-47D cells at 5×10^{-5} mol/l (Figs. 4 and 5).

4. Discussion

The present report shows that the progestin, medrogestone (Prothil®), has a dual effect on the two enzymes involved in the formation and transformation of estrogens in the hormone-dependent MCF-7 and T-47D breast cancer cells: estrone sulfatase and estrogen sulfotransferase, which can regulate the level of estrogen sulfates (ES). Medrogestone significantly decreases the sulfatase activity corresponding to the hydrolysis of the sulfonate group from E_1S and, at the same time, increases the sulfotransferase activity which ensures the reverse reaction, e.g. the sulfonation of E_1. Consequently, the enzymatic controls by medrogestone can contribute to diminishing the cellular estrogenic stimulation in breast cancer.

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 antiestrogen and antigonadotropin. No androgenic or virilization properties have been reported.

In post-menopausal patients E_1S is concentrated in breast cancer tissues and the levels of this conjugate are 10–30 times higher in tumor than in plasma [6]. As E_1S is quantitatively the most important precursor of E_2 in breast cancer tissues, new possibilities can be opened to block E_2 which is originated through this conjugate. Another interesting approach is to increase the conversion of the potent estrogen E_2 in an inactive form by sulfonation, as it is well established that ES have no genomic action. The biologically inactive ES are very important because they can be used as reserve material for the biosynthesis of active E_2 via the sulfatase pathway, as was extensively demonstrated in breast cancer tissues or cell models [17].

The steroid-ST is a broad and complex superfamily including 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 is divided into a phenol sulfating form (P-PST) and a monoamine sulfating form (M-PST) [30–32]. Sulfonation of E_1 and E_2 is specifically done by EST at nanomolar concentrations, whereas P-PST and HST can also act on estrogens, but at micromolar concentrations [33]. In the present experimental conditions, we assayed [^{3}H]-E_1 sulfonation at a physiological concentration of 5×10^{-9} mol/l, which is highly specific for EST activity. Consequently, the hormone-dependent MCF-7 and T-47D cells possess EST activities and the concentrations of [^{3}H]-ES formed, 8.75 and 12.94% respectively, represent an equilibrium position reached over the extended study period (24 h). As the apparent affinities of EST for estrogens are of the same order as those of Kd for the ER (nanomolar concentrations), it was postulated that EST can compete with ER for E_2 binding and abolish the steroid action after processing of ligand-charged ER [34–36]. In support of this hypothesis, it is interesting to remark that a significant homology of sequence was observed between the ligand binding domain of the ER and the putative estrogen-binding domain deduced from bovine placental EST c-DNA [37].

The precise molecular mechanisms responsible for the different dose-dependent effects observed with the progestin, medrogestone, on EST activity remain to be elucidated. However, there have been a substantial number of examples in which a hormone or anti-hormone produced an opposite effect according to its concentration, e.g. the antiestrogen, tamoxifen, on breast cancer cell proliferation is agonist at low doses and antagonist at high doses [38].

There are discrepancies concerning sulfotransferase activities: some authors found only PST or HST activity, but not EST, in the cytosol of hormone-dependent breast cancer cells. However, others report EST and HST activity in MCF-7 and ZR 75-1 breast cancer cells and in mammary tumors [33,39–41]. These variations are probably caused by various factors including cell origin, culture conditions, instability of human EST enzyme and the condition of the enzymatic assays (e.g. cytosol fractions or cell monolayers, in the absence or presence of cofactors). Finally, differences

may be due to the use of cDNAs or recombinant EST expressed either in bacterial or mammalian cell systems [42].

Limited information is available on the regulation of the EST expression in these cells. In a recent paper, we demonstrated that the rate of EST 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. The progestin, promegestone, exerts a dual effect on EST activity in MCF-7 and T-47D breast cancer cells: stimulatory at a low concentration (5×10^{-7} mol/l: +26 and +29%, respectively) and inhibitory at a higher concentration (5×10^{-5} mol/l). These regulatory effects on EST activity were correlated with the level of hEST1 mRNA [28]. In comparison, medrogestone possesses a higher stimulatory effect than promegestone at 5×10^{-7} mol/l, with +53 and +63%, in MCF-7 and T-47D cells, respectively.

The present data establish that the synthetic progestin medrogestone, can reduce the conversion of E_1S to E_2 in both cells (T-47D and MCF-7) by blocking the sulfatase pathway. Despite a higher sulfatase activity in non-treated T-47D cells in culture compared to MCF-7 cells, medrogestone exerts on this enzyme a greater inhibitory effect in the former cell line, even at a low dose (5×10^{-8} mol/l). As T-47D cells express high and functional levels of PR, a progesterone receptor-mediated mechanism in the action of medrogestone could be an attractive aspect to explore.

In previous reports we have found that the activity and/or expression of metabolic enzymes implicated in the formation of active E_2 (e.g. estrone sulfatase, 17β-HSD) can be modulated by various steroid hormones or compounds in breast cancer cells [43–48]. The mechanism by which progestins modify enzymatic activities appear to be complex. Progestins can modulate the activity and/or expression of enzymes at multiple levels; e.g. promegestone can interact directly with sulfatase and regulate the rate of its mRNA in MCF-7 cells [27,49].

In conclusion, the present data demonstrate that the progestin medrogestone, inhibits the sulfatase pathway involved in the biosynthesis of E_2 in hormone-dependent MCF-7 and T-47D breast cancer cells. The same progestin at low doses stimulates the estrogen sulfotransferase activity to form the inactive ES. These combined effects could cause a reduction of the estrogenic environment in breast cancer cells. Clinical trials of these enzyme effects on the formation and transformation of E_2 in breast cancer patients could be the next step to investigate new therapeutic perspectives for the treatment of endocrine-related mammary carcinoma.

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