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Effect of Medrogestone on 17β-hydroxysteroid dehydrogenase activity in the hormone-dependent MCF-7 and T-47D human breast cancer cell lines

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Abstract

Estradiol (E_2) is one of the most important hormones supporting the growth and evolution of breast cancer. Consequently, to block this hormone before it enters the cancer cell, or in the cell itself, has been one of the main targets in recent years. In the present study we explored the effect of Medrogestone (Prothil®) on 17β-hydroxysteroid dehydrogenase (17β-HSD) activities of the hormone-dependent MCF-7 and T-47D human breast cancer cell lines. Using physiological doses of estrone ([^{3}H]-E_1: 5×10^{-9} mol/l) this estrogen is converted in a great proportion to E_2 in both cell lines. After 24 h of the cell culture, Medrogestone significantly inhibits this transformation in a dose-dependent manner by 39% and 80% at 5×10^{-8} M and 5×10^{-5} M, respectively in T-47D cells; the effect is less intense in MCF-7 cells: 25% and 55% respectively. The IC_{50} values are 0.45 μmol/l in T-47D and 17.36 μmol/l in MCF-7 cells.

It is concluded that the inhibition provoked by Medrogestone on the reductive 17β-HSD activity involved in the local biosynthesis of the biologically active estrogen estradiol, may constitute a new therapeutic approach for the treatment of breast cancer.

Keywords: Breast cancer; Medrogestone; 17β-HSD

1. Introduction

The biologically active estrogen, estradiol (E_2), plays an important role in the origin and development of breast cancer [1–3]. Two principal pathways are implicated in the last steps of estradiol formation in breast cancer tissues: the 'aromatase pathway' which transforms androgens into estrogens [4–6], and the 'sulfatase pathway' which converts estrone sulfate into estrone (E_1) [7–11]. Estrone is then transformed into estradiol by the reductive 17β-hydroxysteroid dehydrogenase (17β-HSD; EC: 1:1:1:62) activity [12,13].

It was observed that the 17β-HSD activity in breast cancer depends on the experimental conditions: in vitro studies using human tumor homogenates indicated that the predominant 17β-HSD activity was oxidative rather than reductive [14]. However, in vivo studies, after isotopic infusion of estrogens to postmenopausal breast cancer patients, have shown that the reductive direction ($E_1 \rightarrow E_2$) is greater that the oxidative ($E_2 \rightarrow E_1$) [15].

17β-HSD belongs to a superfamily of enzymes (7 different isoforms are recognized) [16–23], each of which is expressed in specific tissue distribution (substrate and cofactor requirement), and regulation. In previous studies in this laboratory and others, it was observed that in hormone-dependent breast cancer cell lines (MCF-7, T-47D, R-27, ZR-75-1) type I of 17β-HSD was the predominant isoform and acted by converting E_1 to E_2. Other isoforms were also detected as

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type II and IV, which both possess oxidative activities (formation of E_1) [24–27].

Medrogestone (Prothil®) is a synthetic pregnane derivative which has the same physiological activities as progesterone itself and can be used in the treatment of pathological deficiency of the natural hormone. 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 the present paper we report tests on the dose-dependent effects of Medrogestone on the reductive 17β-HSD activity (conver sion of E_1 to E_2) in the human hormone-dependent MCF-7 and T-47D breast cancer cells lines.

2. Materials and methods

2.1. Chemicals

[6,7-^{3}H(*N*)]-Estrone (^{3}H-E_1), (49 Ci/mmol) and [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_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 (Hanover, Germany). The structure of Medrogestone is given in Fig. 1. All chemicals were of the highest purity available.

2.2. Cell culture

The hormone-dependent MCF-7 and T-47D human mammary cancer cell lines were kindly provided by Dr. S.A. 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

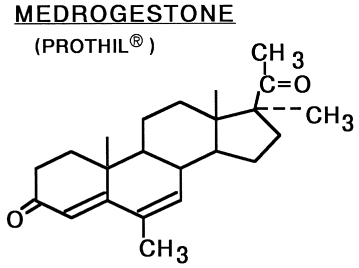

Fig. 1. Structure of the progestin Medrogestone (Prothil®).

(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 weekly. 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. The FCS 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

Preconfluent cells were cultivated in MEM-DCC-FCS (10 ml) with the addition of 5×10^{-9} mol/l of [^{3}H]-E_1 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 were washed twice with ice-cold Hank's balanced salt solution HBSS, (calcium–magnesium free) (A.T.G.C.) 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 measured [28]. After evaporation of the organic phase, the extracts were redissolved in 50 μl of ethanol and the qualitative analysis and quantitative evaluation of E_1 and E_2 were carried out after isolation by TLC on silica gel 60F_{254} plates (Merck, Darmstadt) developed with chloroform–ethylacetate (4:1, v/v) system. [^{14}C]-E_2 (5000 dpm) was added to monitor analytical losses and unlabeled E_1 and E_2 (50 μg) were used as carriers and reference indicators. After visualization of the estrogens under U.V. 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. 3 ml of Opti-fluor (Packard, Rungis) 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_1 to [^{3}H]-E_2, corresponding to the reductive 17β-HSD activity at 24 h, was done by calculating the percentage of the total radioactivity associated with the cells and then expressed as pmol/mg DNA.

2.4. 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.

3. Results

3.1. 17β-Hydroxysteroid dehydrogenase activity in hormone-dependent MCF-7 and T-47D human breast cancer cells: effect of Medrogestone

When a physiological concentration (5×10^{-9} mol/l) of [^{3}H]-E_1 was incubated with the hormone-dependent MCF-7 or T-47D human breast cancer cells in cultures for 24 h at 37°C, a significant transformation of E_1 to E_2 was observed. The reductive activity of 17β-HSD was five times higher in T-47D than in MCF-7 cells. The corresponding values of E_2 formed in the cell compartment were: 3.50 compared to 0.72 pmol/mg DNA, respectively (Table 1).

We have shown that Medrogestone inhibits the conversion of E_1S to E_2 in both MCF-7 and T-47D breast cancer cells [29]. As this metabolism implicates the transformation of E_1 to E_2, it was interesting to explore if Medrogestone exerts an inhibition on the reductive activity of 17β-HSD. As shown in Table 1, when Medrogestone is incubated with a physiological concentration (5×10^{-9} mol/l) of [^{3}H]-E_1 in the MCF-7 or T-47D breast cancer cell cultures for 24 h, this progestin shows a significant inhibition ($p < 0.05$) of the reductive 17β-HSD activity in both cells, and at all the concentrations tested (5×10^{-8} to 5×10^{-5} mol/l)

Table 1
Effect of Medrogestone on the conversion of estrone (E1) to estradiol in hormone-dependent MCF-7 and T-47D human breast cancer cells[a]

	Estradiol (in the cells, pmol/mg DNA)	
	MCF-7 cells	T-47D cells
[^{3}H]-E_1 alone	0.72 ± 0.06	3.50 ± 0.25
+ Medrogestone		
5×10^{-5}	0.32 ± 0.03[c]	0.71 ± 0.04[c]
5×10^{-6}	0.37 ± 0.04[c]	1.35 ± 0.09[c]
5×10^{-7}	0.48 ± 0.04[b]	1.70 ± 0.10[c]
5×10^{-8}	0.54 ± 0.06[b]	2.13 ± 0.16[b]

[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} to 5×10^{-5} mol/l. The calculation of estradiol was carried out after isolation of the hormones as indicated in Section 2. The data represent the average ± S.E.M. of 3–4 duplicate independent experiments.

[b] $p < 0.05$ versus control value (nontreated cells; [^{3}H]-E_1 alone).

[c] $p < 0.01$ versus control value (nontreated cells; [^{3}H]-E_1 alone).

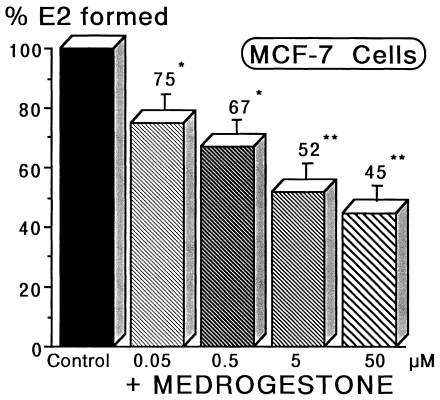

Fig. 2. Effects of Medrogestone (Prothil®) on the conversion of estrone (E_1) to estradiol (E_2) in the hormone-dependent MCF-7 human breast cancer cells. Preconfluent cells were incubated for 24 h at 37°C with a physiological concentration of estrone ([^{3}H]-E_1: 5×10^{-9} mol/l) alone (control: nontreated cells) or in the presence of 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 TLC as indicated in Section 2. The data are the mean ± S.E.M. of duplicate determinations of 3–4 independent experiments. The control values were assigned 100%. *$p < 0.05$ versus control value (nontreated cells; [^{3}H]-E_1 alone). **$p < 0.01$ versus control value (nontreated cells; [^{3}H]-E_1 alone).

(Figs. 2 and 3). This inhibition shows a dose-dependent response, with a higher intensity in T-47D cells: 39–80% at 5×10^{-8} and 5×10^{-5} mol/l, respectively, compared to 25–55% with MCF-7 cells. The IC_{50} values (corresponding to the concentrations of Medrogestone which decrease the formation of E_2 by 50%) indicate that the inhibitory effect is 38 times

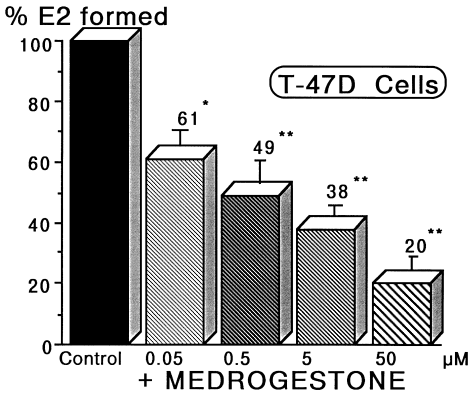

Fig. 3. Effects of Medrogestone (Prothil®) on the conversion of estrone (E_1) to estradiol (E_2) in the hormone-dependent T-47D human breast cancer cells. Preconfluent cells were incubated for 24 h at 37°C with a physiological concentration of estrone ([^{3}H]-E_1: 5×10^{-9} mol/l) alone (control: nontreated cells) or in the presence of 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 TLC as indicated in Section 2. The data are the mean±S.E.M. of duplicate determinations of 3–4 independent experiments. The control values were assigned 100%. *$p < 0.05$ versus control value (nontreated cells; [^{3}H]-E_1 alone). **$p < 0.01$ versus control value (nontreated cells; [^{3}H]-E_1 alone).

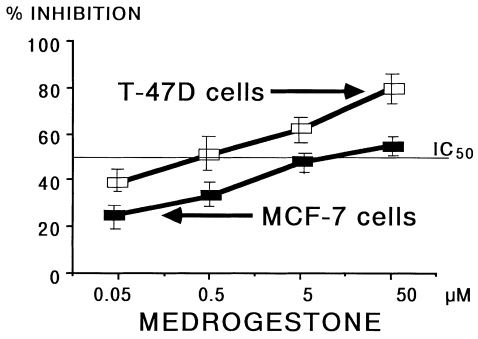

Fig. 4. Inhibitory effects of Medrogestone (Prothil®) on the conversion of estrone (E_1) to estradiol (E_2) in the hormone-dependent MCF-7 and T-47D human breast cancer cell lines. Preconfluent cells were incubated for 24 h at 37°C with a physiological concentration of estrone ([^{3}H]-E_1: 5×10^{-9} mol/l) alone (control: nontreated cells) or in the presence of Medrogestone at a concentration range from 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_1 alone). The values were determined by using nonlinear regression analyses.

more intense in T-47D than in MCF-7 cells: 0.45 µmol/l and 17.36 µmol/l for T-47D and MCF-7 cells, respectively (Fig. 4).

4. Discussion

17β-hydroxysteroid dehydrogenase (17β-HSD) is a widely distributed enzyme implicated in the interconversion of the 17-hydroxy↔17-keto function in steroid hormones, and for estrogens it can be involved in both the inactivation: oxidative activity [estradiol (E_2)→ estrone (E_1)] and reductive for the formation of the potent biological E_2 [30,31]. Previous studies in this laboratory and others have demonstrated that 17β-HSD has a predominant reductive orientation in hormone-dependent breast cancer cells (e.g.: MCF-7 and T-47D) [24,25,32–34]. This observation has led to the exploration of specific inhibitors of the reductive 17β-HSD activity as therapeutic target to diminish the intratumoral formation of E_2 [35,36].

The present data show that Medrogestone (Prothil®), a synthetic pregnane derivative of progesterone, can significantly decrease the formation of E_2 in the hormone-dependent MCF-7 and T-47D human breast cancer cell lines. The inhibitory effect of the reductive 17β-HSD activity is dose-dependent and is more intense, even at low doses, in the T-47D cell line than in the MCF-7 cells. At a low concentration (5×10^{-8} mol/l) the percentage of inhibition is 39±3% for T-47D, compared to 25±2% for the MCF-7 cells. This difference is confirmed by the IC_{50} values: 0.45 µmol/l and 17.36 µmol/l, respectively. As shown with MCF-7 and T-47D cell lines, the variability of the 17β-HSD activity could be explained by the presence of various distinct isoforms (to date I to VII) present in specific target tissues [16–23]. In hormone-dependent breast cancer cells, type I is the principal isoform and exhibits a high specificity for estrogens and preferentially catalyzes the reduction reaction (E_1 to E_2). This type I gene consists of 7 exons and 5 introns that span 6.2 kb and encode two species of mRNA, 1.3 and 2.3 kb. These transcripts are differentially expressed in breast cancer cell lines or tumors. Only the 1.3 kb species is under hormonal regulation, whereas the 2.3 kb mRNA is constitutively expressed [26,37].

The orientation of the enzymatic activity (oxidative or reductive) is also greatly dependent on the nature and concentration of the cofactors [NAD(P) or NAD(P)H] produced by the cells. In the present experiments, no cofactors (e.g. NAD(P)H) were added to the culture medium, so that the physiological biochemical environment was maintained in each type of cell. In contrast, hormone-independent breast cancer cell lines, as well as normal breast cells, favored the formation of E_1 [25,38,39]. These data suggest that estrogen metabolism is closely dependent on the breast tumoral process, and that type I-17β-HSD could be considered an important factor of the estrogen-dependent breast cancer growth.

The regulation of type I 17β-HSD in breast cancer is under investigation, but the implications of progestins, as well as growth factors (IGF-I and -II), cytokines (interleukin-4 and -6) and retinoic acids has been demonstrated [26,27,40–43].

Poutanen et al. [27,40] have observed that the 17β-HSD enzyme protein concentration, as well as the level of its 1.3 kb mRNA, is much higher in T-47D than in MCF-7 cells. Treatment by progestins such as Org 2058 increases the concentration of enzyme protein (type I) and the 1.3 kb mRNA in T-47D cells only.

In previous studies, we have shown that different progestins, such as Org OD14, nomegestrol acetate, have the capacity to inhibit the formation of E_2 from E_1 and the effect is generally more intense with the T-47D cell line [44,45]. Other progestins, such as norethisterone, medroxyprogesterone acetate or levonorgestrel, increase both the oxidative and reductive 17β-HSD activities, whereas promegestone has no effect or stimulates only the oxidative activity [32,40,46].

The present data show that the progestin Medrogestone inhibits the transformation of E_1 to the potent estrogen E_2 in hormone-dependent MCF-7 and T-47D breast cancer cell lines. These results can open new therapeutic possibilities for the control of estradiol in breast tumors.

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