Molecular and Cellular Endocrinology 301 (2009) 142–145

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Estrogen formation in endometrial and cervix cancer cell lines: Involvement of aromatase, steroid sulfatase and 17β-hydroxysteroid dehydrogenases (types 1, 5, 7 and 12)

Michelle-Audrey Fournier, Donald Poirier*

Oncology and Molecular Endocrinology Laboratory, (Medicinal Chemistry Division), CHUQ-CHUL Research Center and Université Laval, Québec, Québec G1V 4G2, Canada

ARTICLE INFO

Article history:
Received 27 June 2008
Received in revised form 28 August 2008
Accepted 29 August 2008

Keywords:
17β-Hydroxysteroid dehydrogenase
Steroid sulfatase
Aromatase
Endometrial cancer
Inhibitor
Enzyme

ABSTRACT

The involvement of aromatase, steroid sulfatase (STS) and reductive 17β-hydroxysteroid dehydrogenases (17β-HSDs) in the production of estrogens was determined in four cell lines of endometrial cancer (Ishikawa, HEC-1A, HEC-1B and RL-95) and one cell line of cervix cancer (Hela) in culture. After incubation with 4-androstene-3,17-dione (4-dione), there are no estrogens, estrone (E1) and estradiol (E2), detected suggesting that the pathway of aromatase is not important in these cell lines. In whole cells, the results show low percentages of transformation of estrone sulfate (E1S) into E1 suggesting that the entrance of E1S is difficult. However, in homogenized cells the STS activity was much higher and fully blocked by an inhibitor. Using selective inhibitors for each reductive 17β-HSD (types 1, 5, 7 and 12), alone or in combination, we did not succeed in completely blocking the conversion of E1 into E2, suggesting that another 17β-HSD (known or unknown) is involved in the formation of E2 from E1.

© 2008 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

The majority of endometrial cancers are estrogen-sensitive and estradiol (E2), the most potent estrogen, is suspected to play an important role in their development. Indeed, the activation of estrogen receptors (ER) by E2 leads to the transcription of genes that can be linked to the proliferation of cancer cells. The inhibition of aromatase, steroid sulfatase (STS) and reductive 17β-HSDs could reduce the concentration of E2 and possibly reduce the spreading of ER^+ tumors. Aromatase is the enzyme that catalyzes the transformation of 4-androstene-3,17-dione (4-dione) into estrone (E1) and testosterone (T) into E2 (Brueggemeier et al., 2005; Smith et al., 2001). STS hydrolyzes the sulfated steroids, abundant in circulation but inactive on steroid receptors, into active hydroxylated steroids. Its main four substrates are dehydroepiandrosterone sulfate, E1S, cholesterol sulfate and pregnenolone sulfate, which are transformed into dehydroepiandrosterone, E1, cholesterol and pregnenolone, respectively (Reed et al., 2005; Smith et al., 2001). The 17β-HSDs regulate the levels of active and inactive steroids through a reduction or an oxidation of their substrate (Poirier, 2003; Smith et al., 2001). The reductive isoforms involved in the transformation of E1 into E2 are 17β-HSDs types 1, 7 and 12 (Luu-The, 2001; Luu-The et al., 2006; Moeller and Adamski, 2006), although 17β-HSD type 5 can also transform E2 from E1 but its major activity is the production of T from 4-dione (Penning et al., 2006; Luu-The, 2001).

The purpose of our study is to determine the involvement of key steroidogenic enzymes, namely aromatase, STS and 17β-HSDs 1, 5, 7 and 12, in the production of potent estrogen E2 in four endometrial (Ishikawa, HEC-1A, HEC-1B and RL-95) and one cervix (Hela) cancer cell lines.

2. Materials and methods

2.1. Cell culture

The HEC-1A, HEC-1B, RL-95 and Hela cell lines were obtained from American Type Culture Collection (ATCC) while the Ishikawa cell line was obtained from European Collection of Cell Cultures (ECACC). The stable HEK-293 cells overexpressing STS were obtained from Dr. Van Luu-The (CHUQ-CHUL Research Center). They were maintained in culture flasks (75 cm^2 growth area; BD Falcon) at 37 °C in presence of 5% CO_2 humidified atmosphere. HEC-1A, Hela and Ishikawa cells were grown in Dubelcco's Modified Eagle's Medium nutrient mixture F-12 ham (DME-F12) medium supplemented with 10% fetal bovine serum (FBS), penicillin (100 IU/mL) and streptomycin (100 μg/mL). The HEC-1B cells were propagated in minimal essential medium (MEM) supplemented with 10% FBS, penicillin (100 IU/mL), streptomycin (100 μg/mL), sodium pyruvate (1 mM) and non-essential amino acids (0.1 mM). The RL-95 cells were grown in DME-F12 medium supplemented with 10% FBS, penicillin

* Corresponding author at: Oncology and Molecular Endocrinology Laboratory (Medicinal Chemistry Division), CHUQ-CHUL Research Center and Université Laval, 2705 Laurier Boulevard, Québec, Québec G1V 4G2, Canada. Tel.: +1 418 654 2296; fax: +1 418 654 2761.
E-mail address: donald.poirier@crchul.ulaval.ca (D. Poirier).

0303-7207/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mce.2008.08.027

(100 IU/mL), streptomycin (100 μg/mL) and insulin (50 ng/mL). HEK-293 cells overexpressing STS were grown in MEM supplemented with 7% FBS, glutamine (2 mM), penicillin (100 IU/mL), streptomycin (100 μg/mL), sodium pyruvate (1 mM), non-essential amino acids (0.1 mM) and geneticin (G418 sulfate) (700 μg/mL) (Gibco, Burlington, ON, Canada).

2.2. Transformation of 4-dione in whole cells

2.2.1. Transformation after 24 h

Each cell line was seeded in 24-well plates (BD Falcon) at different concentrations of cells (0, 500, 1000, 5000, 10,000, 15,000, 20,000, 25,000, 50,000, 75,000, 100,000, 150,000, 200,000, 250,000 and 300,000 cells/well) in triplicate. After 24 h at 37 °C in 1 mL of culture medium, an ethanolic solution of [^{14}C]-4-dione (American Radiolabeled Chemicals, Inc., St. Louis, MO, USA) was added to obtain a concentration of 50 nM in the culture medium and the cells were then incubated at 37 °C for another 24 h. The concentration of ethanol in the culture medium was lower than 0.01% (v/v). At the end of the incubation period, the steroids were extracted with diethyl ether and the solvent evaporated. The residue was dissolved in CH_2Cl_2, spotted on a silica gel plate (TLC, 10 cm × 20 cm × 0.2 mm, Kieselgel 60 F254) and eluted with toluene:acetone (4:1). Metabolites were separated according to their polarity from more polar (low R_f) to less polar (high R_f) steroids: 3α/β-diol (same R_f), T, E2, epi-ADT, ADT/DHT (same R_f), 4-dione, E1 and A-dione. Radioactivity signals were detected, identified by correlation with known standards and quantified using a Storm 860 Imager (GE Health Care, Sunny Vale, CA, USA). The percentage of transformation of [^{14}C]-4-dione into a metabolite (M_n) was calculated as follows: % transformation = {([^{14}C]–M_n)/(Σ of radioactivity associated with all spots)} × 100.

2.2.2. Transformation after 1, 2, 4, 6 and 8 days

HEC-1A, RL-95 and Ishikawa cells were seeded in culture flasks (25 cm^2 growth area; BD Falcon) at 0.3 million cells by flask in triplicate. After 24 h at 37 °C in 8 mL of culture medium, an ethanolic solution of [^{14}C]-4-dione (American Radiolabeled Chemicals, Inc., St. Louis, MO, USA) was added to obtain a concentration of 50 nM in the culture medium and the cells were then incubated at 37 °C for another 24 h. The concentration of ethanol in culture medium was lower than 0.01% (v/v). After 1, 2, 4, 6 and 8 days, 1 mL of medium was removed for extraction and quantification of metabolites and 1 mL of fresh medium was added in the culture flask. As reported in Section 2.2.1, the steroids were extracted, identified and quantified to determine the % of transformation.

2.3. STS activity

2.3.1. Transformation of E1S in whole cells

HEC-1A, RL-95, Hela and Ishikawa cells as well as HEK-293 cells overexpressing STS activity were seeded at 1, 2.5 and 5 million cells in culture flasks (25 cm^2 growth area; BD Falcon). HEC-1B cells were seeded at 1, 2.5 and 3.5 million. After 24 h at 37 °C in 8 mL of medium, an ethanolic solution of [^{3}H]-E1S (PerkinElmer, Boston, MA, USA) was added to obtain a concentration of 9 nM in culture medium and the cells were then incubated at 37 °C for another 24 h. The concentration of ethanol in culture medium was lower than 0.01% (v/v). At the end of the incubation period, an aliquot of medium (1 mL) was removed and added to 1 mL of xylene, and the solution centrifuged at 3000 rpm for 20 min to separate the organic ([^{3}H]-E1) and aqueous ([^{3}H]-E1S) phases. Radioactivity was recorded in 300 μL of each phase with a Wallac 1411 Liquid Scintillation Counter (Turku, Finland) to determine the % of transformation.

2.3.2. Transformation of E1S in homogenized cells

The cells were homogenized by repeated (five times) freezing at −80 °C and defrosting at 4 °C. They were thus disrupted, but cell debris and un-disrupted cells were not separated by centrifugation. This cell preparation was incubated at 37 °C for 2 h (in shaking water bath) with [^{3}H]-E1S (9 nM), adjusted to a final concentration of 1 μM with E1S (Sigma, Poole, Dorset, UK), and tris–acetate buffer at pH 7.4 containing 5 mM EDTA and 10% glycerol. The steroids were quantified as reported in Section 2.3.1 to determine the % of transformation.

2.3.3. Inhibition of the transformation of E1S in homogenized cells

The experimental conditions were the same as reported in Section 2.3.2 except that an ethanolic solution of the STS inhibitor EM-1913 (Ciobanu et al., 2003) was added in the reaction mixture to obtain final concentrations of 0.1, 1 and 10 μM. The final concentration of ethanol in reaction mixture with an inhibitor or without (control) was always the same (0.5%, v/v). The steroids were quantified as reported in Section 2.3.1 to determine the % of transformation and then the % of inhibition.

2.4. 17β-HSD activity

2.4.1. Transformation of E1 in whole cells

Each cell line was seeded in 24-well plates (BD Falcon) at different concentrations of cells (0, 20,000, 25,000, 50,000, 75,000, 100,000, 150,000, 200,000 and 300,000 cells/well) in triplicate. After 24 h at 37 °C in 1 mL of medium, an ethanolic solution of [^{14}C]-E1 (American Radiolabeled Chemicals, Inc., St. Louis, MO, USA) was added to obtain a concentration of 60 nM in the culture medium and the cells were then incubated at 37 °C for another 24 h. The concentration of ethanol in culture medium was lower than 0.01% (v/v). At the end of the incubation period, the steroids were extracted with diethyl ether and the solvent evaporated. The residue was dissolved in CH_2Cl_2, spotted on a silica gel plate and eluted with toluene:acetone (4:1). The steroids were detected according to an established procedure (Tremblay et al., 2005; Cadot et al., 2007) and quantified using a Storm 860 Imager to determine the % of transformation.

2.4.2. Inhibition of the transformation of E1 in whole cells

HEC-1A, HEC-1B and Ishikawa cells were seeded in 24-well plates (BD Falcon) at 200,000 cells/well in triplicate whereas Hela cells were seeded at 300,000 cells/well. The experimental conditions were the same as reported in Section 2.4.1 except that an ethanolic solution of a selective inhibitor of each 17β-HSD (type 1, 5, 7 or 12) or a combination of different inhibitors (INH-1, INH-5, INH-7 or INH-12) was added in the culture medium to obtain a final concentration of 10 μM. These inhibitors were selected from previous work performed in our laboratory during recent years (Laplante et al., 2008; Bydal et al., in press; Bellavance et al., 2004; Poirier, 2003) and found to be selective for each targeted 17β-HSD isoform (Laplante et al., in press). The final concentration of ethanol in culture medium with inhibitors or without (control) was always the same (0.5%, v/v). According to an established procedure (Section 2.4.1; Tremblay et al., 2005; Cadot et al., 2007), steroids E1 and E2 were extracted and quantified to determine the % of transformation and then the % of inhibition.

3. Results and discussion

We first verified the presence of aromatase activity by incubating each cell line for 24 h in presence of 4-dione. Surprisingly, we did not observe any formation of estrogens E1 and E2 using standard TLC analysis, but we detected a series of other metabolites. Thus, with the HEC-1B cell line as a representative example (Fig. 1), the quantity of A-dione increased to ∼20% and then decreased to ∼10% whereas the radioactivity associated to ADT/DHT gradually increased to reach 45%. The concentrations of other metabolites 3α/3β-diol, T and epi-ADT just slightly increased but remain lower than 10%. We therefore selected the three estrogen-receptor positive (ER$^+$) cell lines, namely HEC-1A, RL-95 and Ishikawa, to carry out longer incubation periods (2, 4, 6 and 8 days). Even after 8 days, there is still no E1 and E2 detected (Table 1), suggesting that the pathway of aromatase is not important within these cell lines. Since the estrogens could come from other tissues, we turned our attention to the pathway of STS. Indeed, E1 can be recovered from E1S after the enzymatic action of STS. We therefore checked the rate of transformation of E1S into E1 in the five selected cell lines and in

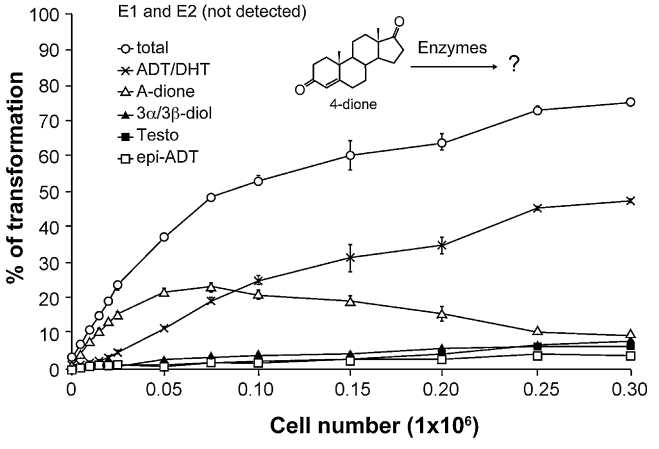

Fig. 1. Transformation of [^{14}C]-4-dione (50 nM) into its [^{14}C]-metabolites after 24 h of incubation in HEC-1B whole cells. ADT/DHT: androsterone or/and dihydrotestosterone, A-dione: 5α-androstane-3,17-dione, 3α/3β-diol: 5α-androstane-3α,17β-diol or/and 5α-androstane-3β,17β-diol, Testo: testosterone and epi-ADT: epi-androsterone. This figure is representative of other cell lines (HEC-1A, RL-95, Hela and Ishikawa). The data are the mean (±S.D.) of a triplicate. When the error bars are not shown, they are smaller than the symbol.

Download English Version:

https://daneshyari.com/en/article/2197369

Download Persian Version:

https://daneshyari.com/article/2197369

Daneshyari.com