Ishikawa, HEC-1-A, and ECC-1 Human Endometrial Cancer Cell Lines Display Aryl Hydrocarbon (Ah) Responsiveness

Mark Wormke, Emely Castro-Rivera, and Stephen Safe*

*From the Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, Texas 77843-4466

Abstract

Ishikawa, HEC-1-A, and ECC-1 human endometrial cancer cell lines were evaluated for aryl hydrocarbon (Ah) receptor functionality. The nuclear Ah receptor was detected in Ishikawa and Hec-1-A cell lines by sucrose density gradient analysis using nuclear extracts from cells treated with [^{3}H]TCDD, and the calculated sedimentation coefficients were comparable with Ah receptor values reported for other human cells. Significant increases in CYP1A1-dependent ethoxyresorufin O-deethylase (EROD) activity was observed in all cell lines after treatment with TCDD. TCDD also induced reporter gene activity in Ishikawa and ECC-1 cells transiently transfected with an Ah-responsive plasmid. These preliminary results indicate that endometrial cell lines express functional Ah receptors

Introduction

Endometrial cancer is the most common malignancy of the female genital tract and is the fifth leading cancer in women (1). Over 150,000 new cases are diagnosed worldwide each year. Endometrial cancer incidence increased significantly in the United States during the period from 1965 to 1975; however, levels then decreased and have been relatively constant since 1980. Nevertheless, among all cancer in women, the incidence of uterine cancer in the United States is number 4. Epidemiological studies have identified several risk factors for endometrial cancer and these include: obesity, early age at menarche, later age at menopause, parity and various forms of steroid hormone treatment or use. This profile of risk factors is comparable to those reported for breast cancer and supports an etiologic role for unopposed estrogens in the development of endometrial cancer (2). Cigarette smoking has been linked to an increased incidence of several diseases; however, there is considerable evidence which suggests that cigarette smoking is antiestrogenic. For example, smoking decreases the age of menopause, increases the incidence of osteoporosis, and decreases the risk of benign breast cancer, and other estrogen-linked uterine diseases. Although the antiestrogenic effects of cigarette smoking in breast cancer are equivocal, several studies reported a lower incidence of endometrial cancer in smokers (3,4). Polynuclear aromatic hydrocarbons (PAHs) which are present in cigarette smoke are antiestrogenic in human breast cancer cells. Previous studies in the rodent/uterus mammary and in human breast cancer cells have confirmed the antiestrogenic (and antitumorigenic) activities of structurally diverse Ah receptor agonists and mechanistic studies have confirmed interactions between the Ah receptor and estrogen receptor (ER) signaling pathways (5). Research in this laboratory is focused on development of Ah receptor-based antiestrogens which are effective for clinical treatment of mammary and endometrial cancer. This report summarizes results of initial studies on characterizing the Ah- and estrogen- responsiveness of 3 endometrial cancer cell lines which will be utilized as models for future studies.

Materials and Methods

Cell Maintenance: Ishikawa, HEC-1-A and ECC-1 human endometrial cancer cell lines were maintained in DME/F12 media (Sigma D-8900) supplemented with 2.2 g/l sodium bicarbonate (Sigma), 5% fetal bovine serum (Intergen lot # NB99505), and 10 ml/l antibiotic-antimycotic solution (Sigma A-9909) pH 7.4. Cells were grown in 150-cm^2 culture flasks in an air:carbon dioxide (95%:5%) atmosphere at 37°C.

Preparation of Nuclear Extracts and Determination of Ah Receptor Levels: Cells were harvested by trypsinization (1% trypsin soln.), centrifuged for 5 min at 200 x g, resuspended in 10 ml of culture media (in 50 ml conical tubes), and treated with 10 nM [^{3}H]TCDD for 2 h in a 37 C shaking water bath. Baselines for AhR were obtained by co-treatment with a 200-fold molar excess of non-radiolabeled TCDD. Following the 2 h incubation cells were centrifuged in a Beckman GH-3.7 rotor at 1000 rpm (200 x g) for 5 min at 4 C washed once in 15 ml of ice cold HEGD buffer (25 mM HEPES, 1.5 mM EDTA, 1 mM dithiothreitol, and 10% glycerol, pH 7.6) and repelleted. The pellet was then resuspended and incubated for 10 min on ice in HED buffer (HEGD without glycerol) with intermitent swirling. The cells were then centrifuged for 10 min at 4 C in same rotor at 2000 rpm (900 x g), and the pellet was homogenized using a Wheaton Potter-Elvehjem tissue grinder on ice. The homogenate was centrifuged for 10 min at 4 C in same rotor at 3000 rpm (2000 x g). The pellet was resuspended in an equal volume of HEGD buffer containing 0.5 M potassium chloride and incubated for 1 h on ice with intermitent swirling. The suspension was then centrifuged for 30 min at 4 C in a Beckman 50.2Ti rotor at 33,000 rpm (132,000 x g) to obtain high salt nuclear extract supernatant. 300 ug aliquots of radiolabeled AhR nuclear extracts were layered onto linear sucrose gradients (5 - 25%) prepared in TEG buffer (0.25 mM Tris, 1.5 mM EDTA, 10% glycerol) containing 0.4 M potassium chloride. Following centrifugationin a Beckman Vti 65.2 rotor at 65,000 rpm (385,000 x g) for 2.5 h the gradients were fractionated into 30 equal fractions, and the radioactivity in each fraction was determined by liquid scintillation counting. Specific binding (fmol receptor/mg protein) was calculated by subtracting the total radioactivity under the peak from the baseline (nonspecific binding) and using the specific activity of the radiolabeled ligands. Sedimentation coefficients were calculated using a [^{14}C]BSA standard.

Ethoxyresorufin O-Deethylase (EROD) Activity: Cells were seeded into 100mm tissue culture plates in DME/F12 maintainence media The following day cells were treated with dimethyl sulfoxide (DMSO) vehicle or 10 nM TCDD for 24 h. Cells were harvested by scraping, centrifuged in a Beckman GH-3.7 rotor at 1000 rpm (200 x g) for 5 min at 4°C, and resuspended in Tris-sucrose buffer (38 mM Tris, 0.2 M sucrose, pH 8.0). Aliquots of the cell suspension were used for determining EROD activity by methods previously described (6).

Plasmid Transfection Studies: The pRNH11c plasmid construct which contains the human CYP1A1 regulatory region (-1142/+2434) fused to the bacterial chloramphenicol acetyl transferase (CAT) gene was kindly provided by Dr. R. N. Hines (7). Cells were seeded into 100mm tissue culture plates in DME/F12 maintainence media. The following day each plate was transfected with 10 ug of plasmid DNA using calcium phosphate mediated transfection (8) for 12 h. The media was then changed, and the cells were dosed with DMSO vehicle and 10 nM TCDD and 17b-estradiol. After 48 h cells were washed once with phosphate-buffered saline (PBS) and harvested by scraping. cells were lysed by freezing, thawing, and vortexing 3x using liquid nitrogen, a 37 C water bath, and a vortexer. Cell lysate from each plate was collected and protein was quantitated using the Bradford method (9). Equal protein concentrations from each sample were used to determine CAT activity as previously described (8). Following thin layer chromatography (TLC) the acetylated products were visualized by autoradiography and quantitated using a Betagen Betascope 603 Blot Analyzer.

Statistical Analysis: The statistical differences between groups were determined by ANOVA and Scheffe's test for significance. The data are presented as means ± S.D. Three determinations were carried out for each data point.

Results and Discussion

The Ah-responsiveness of Ishikawa, HEC-1-A, and ECC-1 endometrial cancer cell lines was investigated using several characteristic assays including; TCDD-induced reporter gene activity, induction of EROD activity by TCDD, and sucrose density gradient analysis of nuclear extracts from cells treated with [^{3}H] TCDD. The results summarized in Table 1 show that TCDD induced reporter gene activtiy in Ishikawa and ECC-1 cells transiently transfected with the Ah-responsive pRNH11c plasmid containing the CYP1A1 regulatory region (-1142/+2434) by 6.4-fold and 8.9-fold respectively. Inducibility in HEC-1-A cells is currently in progress. The results in Figure 1 show that TCDD caused a 3.1-, 21-, 17-fold increase in EROD activity in Ishikawa, HEC-1-A, and ECC-1 cells respectively. The results in Figure 2 show that the nuclear Ah receptor was detected in both Ishikawa and HEC-1-A cells on sucrose density gradient analysis using nuclear extract from cells treated with [^{3}H] TCDD; the calculated sedimentation coefficients of 6.0 (Ishikawa) and 6.6 (HEC-1-A)S are consistent with reported Ah receptor values calculated for other human cell lines(10). Expression of the Ah receptor complex in all three endometrial cancer cell lines was also confirmed by gel mobility shift assay using a [^{32}P]DRE probe (data not shown). Results of preliminary studies also show that all 3 cell lines express a functional estrogen receptor and 17β-estradiol induces proliferation of these endometrial cancer cells. Future studies on the antiestrogenic activity of different structural classes of Ah receptor agonists in endometrial cancer cell lines are currently being investigated.

Table 1. *Induction of CAT activity in Ishikawa and ECC-1 endometrial cells transiently transfected with the Ah-responsive RNH11c reporter construct* The cells were transfected with 10 ug of plasmid/treatment group as described in Materials and Methods, and treated with DMSO or TCDD (10 nM) for 48 h. The results are expressed as means ± S.D. for three separate determinations.

Treatment (concentration)	CAT activity (% conversion)
Ishikawa cells	
DMSO	3.8 ± 0.40
TCDD (10 nM)	24.5 ± 2.2
ECC-1	
DMSO	1.7 ± 0.06
TCDD (10 nM)	14.9 ± 1.6

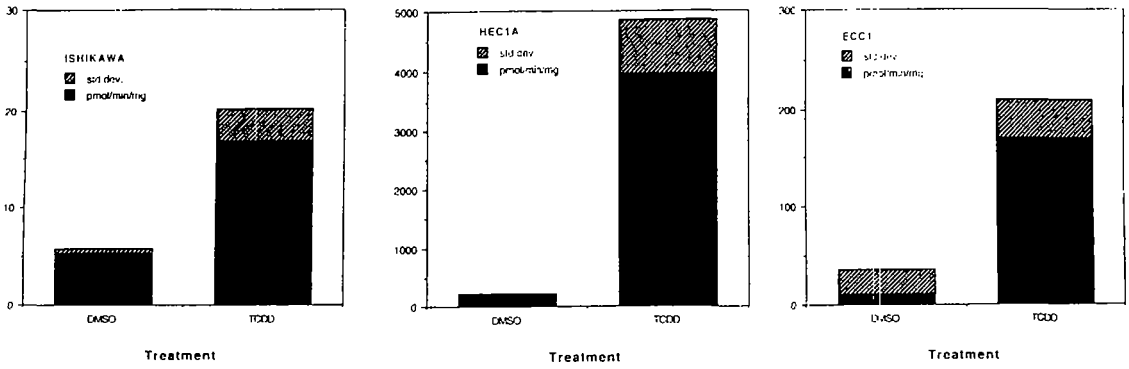

Figure 1. *Induction of EROD activity in Ishikawa, HEC-1-A and ECC-1 endometrial cells.* The cells were dosed with DMSO or 10 nM TCDD for 24 h. Results are expressed as means ± S.D. for three separate determinations in units of pmol/min/mg resorufin product.

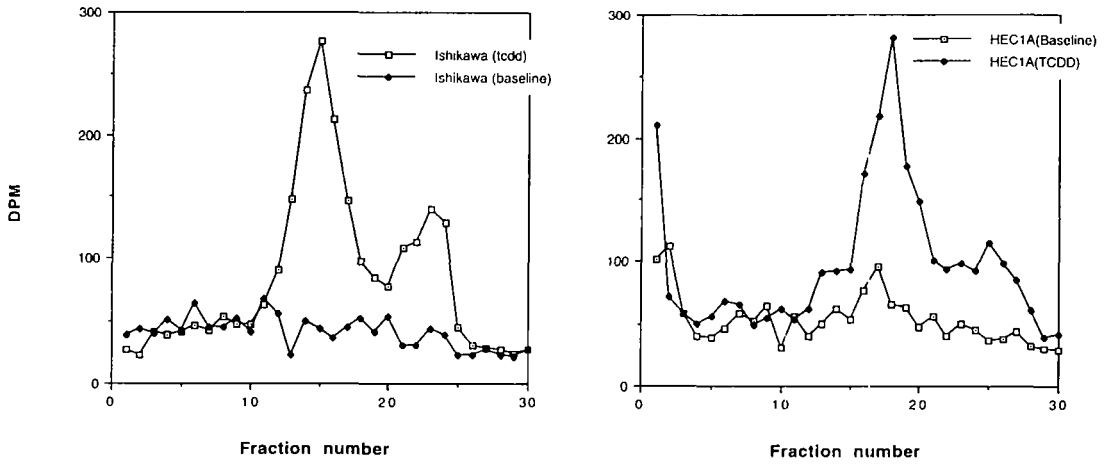

Figure 2. *Velocity sedimentation analysis of the nuclear Ah receptor complex in Ishikawa and HEC-1-A cells after treatment with 10 nM [^{3}H]TCDD.* The cells were harvested, and nuclei were isolated, extracted, and analyzed by velocity sedimentation analysis as described under "Material and Methods".

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