Characterization of the Aryl Hydrocarbon Receptor and Aryl Hydrocarbon Responsiveness in Human Ovarian Carcinoma Cell Lines

C. Rowlands, V. Krishnan, X. Wang, M. Santostefano, S. Safe,2 W. R. Miller, and S. Langdon

Department of Veterinary Physiology and Pharmacology, Texas A & M University, College Station, Texas 77843-4466 1C. R., V. K., X. W., M. S., S. S.I. and ¡CRF Medical Oncology Unit, Western General Hospital, Crewe Road, Edinburgh, EH4 2XU Scotland 2W. R. M., S. L.

ABSTRACT

The human ovarian carcinoma cell lines PE01, PE04, and PE06 express the estrogen receptor and studies with the PE04 cells have shown that tamoxifen inhibits 17β-estradiol-induced proliferation. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a broad spectrum antiestrogen which works through the aryl hydrocarbon receptor. Incubation of the three cell lines with [3H]TCDD followed by isolation of nuclear extracts showed that the PE01, PE04, and PE06 cells express the aryl hydrocarbon receptor (23 to 87 fmol/mg protein) which exhibits sedimentation properties (7.5 to 7.9 S) on sucrose gradients similar to that observed in other mammalian species. Aryl hydrocarbon responsiveness was determined by the induction of P450AI mRNA levels and ethoxyresorufin O-deethylase activity by TCDD. Induction of both parameters was observed only in the PE04 cells. Gel mobility shift assays with a consensus dioxin-responsive element (DRE, 24-mer) showed that after incubation of the nuclear extracts from the 3 cell lines with 32P-DRE a retarded band formed only with nuclear receptor complex from PE04 cells. 17β-Estradiol stimulated proliferation of the PE04 and PE06 but not the PE01 cells; 1 nm TCDD alone either did not affect or inhibited the growth of these cells and 1 nm TCDD significantly inhibited the 17β-estradiol-induced proliferation of the PE04 and PE06 cells. Treatment of the PE04 cells with 1 nm 17β-estradiol resulted in a time-dependent enhanced secretion of the Mr, 52,000 protein (procathepsin D) and, after 48 h, a 51% increase in the secretion of this protein was observed. Cotreatment of the PE04 cells with 0.1 or 1.0 nm TCDD completely inhibited the 17β-estradiol-induced secretion of the Mr, 52,000 protein. These data show that TCDD exhibits antiestrogenic activity in estrogen receptor-positive ovarian carcinoma cell lines; however, in the PE06 cells, there was no correlation between the effects of TCDD on the induction of CYPIAI gene expression and the results of the gel shift assay (i.e., nonresponsiveness) versus the observed antiestrogenic activity.

INTRODUCTION

Recent studies have demonstrated that the environmental toxin TCDD3 exhibits a broad spectrum of antiestrogenic activities in rodent uterus and human breast cancer cell lines (1). For example, in ER-positive human breast cancer cells, TCDD down-regulates the nuclear ER and inhibits 17β-estradiol-induced cell proliferation, [3H]thymidine uptake, progesterone receptor levels, and the secretion of tissue plasminogen activator activity and the Mr 34,000, 52,000, and 160,000 proteins (2–5). The effects of TCDD are dependent on the presence of a functional Ah receptor which acts as a nuclear ligand-responsive transcription factor (1, 6).

Several studies have described the isolation and characterization of human ovarian carcinoma cell lines and their use as models for understanding the factors which regulate their growth (7–10). Langdon et al. (11, 12) have previously described several cell lines produced from 4 patients with ovarian adenocarcinoma. These ovarian cell lines varied with respect to several parameters including tumor site, previous treatment of the donors, passage number, ER status, and their response to both 17β-estradiol and the antiestrogen tamoxifen. Three of these cell lines, namely PE01, PE04, and PE06, were derived from peritoneal ascites from the same patient and contained relatively high levels of the ER (96, 112, and 132 fmol/mg protein, respectively). Using the PE04 cells as a model, it was also shown that the antiestrogen tamoxifen inhibited 17β-estradiol-induced cell proliferation and uptake of [3H]thymidine into cellular DNA (12). In contrast, the ER-negative ovarian cells were resistant to the effects of both 17β-estradiol and tamoxifen. The differential responsiveness to estrogens and antiestrogens in ER-positive and -negative ovarian cells is parallel to similar effects observed in human breast cancer cell lines (13–16).

This study characterizes the Ah responsiveness of the PE01, PE04, and PE06 ovarian carcinoma cell lines and investigates the antiestrogenic activity of TCDD in these cells.

MATERIALS AND METHODS

Materials. All chemicals and cell culture media were purchased from Sigma Chemical Company (St. Louis, MO) unless otherwise specified. Fetal calf serum was purchased from Hazelton Biologies, Inc. (Lenexa, KS). Plastic cell culture supplies were purchased from Corning Science Products (Corning, NY). [3H]TCDD, TCDD, and TCDF were synthesized in this laboratory. The pretreatment solution buffer was obtained from Integrated Separation Systems (Hyde Park, MA).

Cell Culture Maintenance and Growth. Human ovarian adenocarcinoma cells were isolated by Langdon et al. (11, 12), Imperial Cancer Research Fund, Edinburgh, United Kingdom, as described. All cells were grown at 37°C and 95% humidity in an atmosphere of 5% CO2. Stock cells were grown in Dulbecco’s modified Eagle’s medium/F-12:1 (1:1) Ham’s medium supplemented with 5% fetal calf serum, 1.2 g/liter sodium bicarbonate, and 1× antibiotic-antimycotic solution (100 units penicillin, 100 µg streptomycin, and 0.25 µg amphotericin B/ml media). Cells were passed twice a week by trypsinizing and passing through a 10-ml pipet rapidly 4 times. The cells were cultured according to the methods of Van Zoelen et al. (17). Cells were grown in Dulbecco’s modified Eagle’s medium/F-12:1 (1:1) Ham’s medium without phenol red supplemented with 5% mitogen-free serum, 1.2 g/liter sodium bicarbonate, 1× antibiotic-antimycotic, 0.2% (w/v) bovine serum albumin, 30 µM Na3SeO4, and 10 µg/ml transferrin. The cells were seeded in 6-well plates with 3 wells/treatment group. PE01 and PE04 cells were seeded at 2.6 × 104 cells/well; PE06 cells were seeded at 6 × 104 cells/well. Media were changed every 2 days, and on days 4, 6, and 8, the cells were treated briefly with 1 ml trypsin/EDTA solution and then counted on a Neubauer hemocytometer. Each time fresh medium was added to the cells, the appropriate chemical or hormone in 0.1% DMSO was also added to the culture medium.

In Situ Formation of Nuclear Ah Receptor Complexes. Confuent cultures were washed in PBS, trypsinized, and resuspended in 10 ml culture medium in 25-cm² flasks at a cell concentration about 3 x 10⁶ cells/ml. [3H]TCDD (10 nm) in DMSO was added to the cell culture flasks so that the final concentration of DMSO in the culture medium was not greater than 0.5%. Nuclear extract baselines were obtained by cotreatment with a 200-fold excess of TCDF. The flasks were incubated by gentle shaking for 2 h at 37°C. After...
incubation, the suspended cells were decanted into 50-ml centrifuge tubes and centrifuged at 1000 × g. This and all subsequent procedures were performed at 4°C.

**Preparation of Nuclear Extracts.** Pelleted cells were washed twice in 30 ml of HEGD buffer. The washed cell pellet was resuspended in 1 ml of HED buffer (same as HEGD buffer without the glycerol) and incubated for 10 min on ice. Cell suspensions were transferred to a 2-ml homogenizing tube with an additional 1 ml HEGDM buffer (same as HEGD buffer plus 20 mM sodium molybdate) and homogenized using a tight Teflon pestle/drill apparatus. The homogenate was transferred to a centrifuge tube, 1 ml HEGDM buffer was added, and the tube was centrifuged at 4000 × g for 10 min. The resulting pellet was washed twice with 30 ml of HEGDM buffer, then resuspended in 3 ml of HEGDM buffer containing 0.5 M potassium chloride (pH 8.5), and allowed to stand at 4°C for 1 h. Nuclei prepared in this method were found to be intact and appeared to be greater than 90% free of extranuclear cellular component contamination, as determined by microscopic examination.

**Sucrose Density Gradient Analysis of Nuclear Extracts.** Unbound and loosely bound $[^{3}H]_{TCDD}$ was removed by adding dextran-coated charcoal pellets (1 ml of 0.01:0.001% charcoal:dextran per ml of nuclear extract) to the nuclear extract. The dextran-coated charcoal was resuspended using a Vortex mixer, and the sample was incubated for 10 min at 4°C. The dextran-coated charcoal was removed by centrifugation at 4000 × g × 10 min at 4°C. Aliquots (300 µl) of sample were layered onto linear sucrose gradients (5–25%) prepared in HEG buffer containing 0.4 M potassium chloride. Gradients were centrifuged at 4°C for 2.5 h at 404,000 × g. After centrifugation, 30 fractions were collected from each gradient, and radioactivity in each fraction was determined by liquid scintillation counting and corrected for counting efficiency.

**EROD Activity.** Trypsinized cells were plated into 25-cm² tissue culture flasks (10⁵ cells/ml), allowed to attain 60% confluency, and treated with the appropriate concentration of TCDD for 24 h. Cells were harvested by trypsinizing, centrifuging the cells at 1000 × g for 5 min at 4°C, and washing once in PBS. Cells were resuspended in 400 µl Tris-sucrose buffer (38 mM Tris-HCl, 0.2 M sucrose, pH 8.0). Aliquots of the cells (50 µl) were incubated with the addition of 1.15 ml cofactor solution (containing 1 mg bovine serum albumin, 0.7 mg NADH, 0.7 mg NADPH, and 1.5 mg MgSO₄ in 0.1 M 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid buffer, pH 7.5) in a 37°C water bath for 2 min and the reaction was started by adding 50 µl ethyroyIatin (1 mg ethyroyIatin/40 ml methanol) and incubating for 15 min. The reaction was stopped by adding 2.5 ml methanol. Samples were centrifuged for 10 min at 1000 × g. The supernatant was used for fluorescence measurement at an excitation wavelength of 550 nm and an emission wavelength of 585 nm.

**P4501A1 mRNA Levels.** CYP1A1 mRNA levels were measured using a 1.2-kilobase PstI fragment of the rat P450c cDNA kindly provided by Dr. Alan Anderson (Laval University, Quebec City, Quebec, Canada). β-Tubulin mRNA levels were measured using a 1.3-kilobase EcoRI fragment of mouse β-tubulin cDNA (a gift from Dr. Masahito Negishi, National Institute of Environmental Health Sciences, Research Triangle Park, NC). Total RNA was isolated by the guanidinium thiocyanate/acid phenol extraction method (18). Ten µg of total RNA were separated on a 1.2% agarose/1% formaldehyde gel in 20 mM sodium phosphate-2 mM EDTA, transferred onto nylon membrane by capillary action, and bound to the membrane by UV cross-linking. The cDNAs were labeled with [α-3²P]dCTP using a Random Primers DNA Labeling System (BRL) and annealed. The oligonucleotide was labeled at the 5' end using T4-polynucleotide kinase and [γ-3²P]ATP. DNA binding was measured using a gel retardation assay (19). Five µl of the nuclear extract (2 mg protein/ml) from the control (DMSO) and TCDD-treated cells were incubated in HEDG buffer with 200 ng polydextroseinosinate-polyoxyoctylyeldate for 15 min at 20°C. Following the addition of [³²P]-labeled DNA, the mixture was incubated for an additional 15 min at 20°C. Reaction mixtures were loaded onto a 5% polyacrylamide gel (acylamide:bisacrylamide, 30:0.8) and electrophoresed at 110 V in 0.9 mM Tris-borate and 2 mM EDTA, pH 8.0. Gels were dried and protein-DNA interactions determined by autoradiography.

**Determination of Secreted M, 52,000 Protein.** MCF-7 human breast cancer cells were grown as described previously (20). After initial attachment and growth of MCF-7 cells for 5 days or PE04 cells for 2 days, the cells were washed with phosphate-buffered saline and treated with fresh media containing the appropriate chemicals. This procedure was then repeated after 24 h; 18 h later the cells were washed with phosphate-buffered saline and the medium was replaced with Dulbecco’s modified Eagle’s medium/F-12 containing the essential amino acids along with the respective chemicals for a total volume of 750 µl. Serum and bovine serum albumin were not used since they interfered with the analysis for the M, 52,000 protein. At different time points, the medium was removed and spun in an Eppendorf centrifuge at 10,000 rpm for 2 min; 25 µl of the supernatant were then used for protein determination and the remaining solution was used for electrophoretic analysis. An aliquot (10 µg) of the secreted proteins was mixed with the sample buffer containing SDS, β-mercaptoethanol, glycerol, Tris, and bromophenol blue to a total volume of 30 µl as described (20). The samples were heated for 5 min in a boiling water bath and 30 µl of each sample were loaded onto a 1.5-mm-thick 12% acrylamide slab gel (containing 0.075% bisacrylamide) with a 3% stacking gel (containing 0.15% bisacrylamide). The gels were run at 75 V (constant voltage), then washed in 12% trichloroacetic acid solution overnight, and incubated in the pretreatment solution for 60 min followed by 60 min of staining in the ISS ProBlue staining solution. The gels were then washed (5 times) for 2 min with deionized water followed by fixing in a 50% methanol-10% acetic acid solution for 30 min, and fixation was continued overnight in a 5% methanol-7% acetic acid solution. The gels were treated with 10% glacial acetic acid solution for 30 min and washed (4 times) with deionized water. The gels were then stained with the ammoniacal silver stain solution for 15 min and washed (5 times) with deionized water. The bands were developed using a developing solution containing 0.5 g sodium citrate and 0.5 ml 37% formaldehyde solution in 500 ml deionized water. The stain was fixed using the Kodak Rapid Fix solution, washed with deionized water, and dried; the band intensities on the gel were quantitated using a Soft Laser Scanning Densitometer (Molecular Dynamics 300A). The percentages of the area of the scans due to the respective proteins were then compared with controls and treated samples.

**Statistical Analysis.** All the experiments were carried out in triplicate or quadruplicate and the results are expressed as means ± SD. Statistical significance was determined by performing analysis of variance using Scheffe’s test.

**RESULTS**

**Characterization of the Ah Receptor.** The ovarian adenocarcinoma cell lines were treated with 10 nm $[^{3}H]_{TCDD}$ or 10 nm $[^{3}H]_{TCDD}$ plus 2000 nm unlabeled 2,3,7,8-TCDF for 2 h in suspension, the nuclei were isolated by differential centrifugation and the nuclear extracts were analyzed by velocity sedimentation analysis (Fig. 1). The sedimentation coefficients for the specifically bound nuclear Ah receptor peak were observed at 7.5 ± 0.06, 7.8 ± 0.02, and 7.9 ± 0.10 S for the PE01, PE04, and PE06 cells, respectively; the concentrations of the nuclear Ah receptor in these ovarian cells treated with 10 nm $[^{3}H]_{TCDD}$ were 23 ± 0.6, 87 ± 4.3, and 73.3 ± 6.4 fmol/mg protein, respectively.

**Induction of EROD Activity and P4501A1 mRNA Levels and Gel Retardation Assay.** The concentration-dependent induction of EROD activity by TCDD was also determined in the PE01, PE04, and PE06 cell lines (Table 1). Significant induction was obtained in the PE04 cell line and the maximum induced EROD activity was 16.6 ± 0.7 pmol/min/mg protein which was observed at a TCDD concentration of 10 nm. In contrast, TCDD did not induce EROD activity in the PE01 or PE06 cells. The results in Table 2 summarize the effects of 10 nm TCDD on the steady state P4501A1 mRNA levels in PE01, PE04, PE05, PE06, and PE08 cell lines.
Cell Proliferation. The results in Fig. 3 summarize the effects of DMSO (control), 1 nm 17β-estradiol, 1 nm TCDD, and 1 nm 17β-estradiol plus 1 nm TCDD on the proliferation of PE01, PE04, and PE06 cells over a period of 8 days. In PE01 cells, there were no significant differences in the rate of cell proliferation between any of the treatment groups. Treatment with 17β-estradiol resulted in a 134% increase in the proliferation of PE04 cells after 8 days whereas TCDD caused a 44% decrease in growth. In the cotreatment studies, TCDD significantly inhibited the 17β-estradiol-induced proliferation of PE04 cells. In the PE06 cells, 17β-estradiol caused a 62% increase in cell growth (compared to control cells). TCDD alone slightly inhibited the

Table 1 Concentration-dependent induction of EROD activity by TCDD in human ovarian carcinoma cell lines

<table>
<thead>
<tr>
<th>Concentration (nm)</th>
<th>PE01</th>
<th>PE04</th>
<th>PE06</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>nd</td>
<td>7.9</td>
<td>7.6</td>
</tr>
<tr>
<td>1</td>
<td>nd</td>
<td>16.6</td>
<td>7.6</td>
</tr>
<tr>
<td>0.1</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>0.01</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>DMSO (0.1%)</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

* nd, no detectable activity.
* Significantly induced (p < 0.01) compared to control (DMSO)-treated cells.

Table 2 Induction of P4501A1 mRNA levels in human ovarian carcinoma cell lines

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PE01</th>
<th>PE04</th>
<th>PE06</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>148 ± 19.5</td>
<td>173 ± 26.6</td>
<td>161 ± 33.4</td>
</tr>
<tr>
<td>TCDD (10 nm)</td>
<td>167 ± 21.1</td>
<td>314 ± 17.4</td>
<td>211 ± 33.8</td>
</tr>
<tr>
<td>TCDD (100 nm)</td>
<td>166 ± 38.9</td>
<td>304 ± 59.1</td>
<td>204 ± 39.5</td>
</tr>
</tbody>
</table>

* Significantly higher (P < 0.01) than the DMSO-treated PE04 cells.

and PE06 cells. Significant (<2-fold) induction of P4501A1 mRNA was observed only in the PE04 cell line although some increased levels were also noted in PE06 cells. The results in Fig. 2 summarize the gel mobility shift assays of nuclear extracts from the 3 cell lines after treatment with the solvent (DMSO) or 10 nm TCDD. Two constitutive retarded bands of equal intensity were observed for extracts from all 3 cell lines treated with either DMSO or TCDD. In contrast, an intense TCDD-induced retarded band (see indicator bracket in Fig. 2) was observed for nuclear extracts from the PE04 cell whereas this band was present but not induced in the PE06 cells. Relatively low levels of the induced band were also noted in the PE01 cells.
growth of PE06 cells whereas in the cotreatment studies TCDD significantly inhibited 17β-estradiol-induced cell proliferation (Fig. 3).

Secretion of the M, 52,000 Protein (Procathepsin D). The effects of the different treatments on the secretion of the M, 52,000 protein (procathepsin D) was determined in the PE04 cell lines (Table 3) and the levels of secreted protein were compared to those observed in MCF-7 human breast cancer cells (the value for this cell line was set at 100%). The concentrations of the M, 52,000 protein were determined by SDS-polyacrylamide gel electrophoresis separation, followed by double staining and densitometric analysis (20). PE04 cells (untreated) secreted 109% higher levels of the M, 52,000 protein than MCF-7 cells; in addition, there was a time-dependent increase in the secretion of this protein in PE04 cells treated with 1 nM 17β-estradiol. No significant increase in the 17β-estradiol-induced secretion of the M, 52,000 protein was observed 6, 12, and 24 h after addition of the hormone whereas the secretion was significantly increased after 36 and 48 h. Treatment of PE04 cells with 0.1 and 1.0 nM TCDD caused a concentration-dependent decrease in the secretion of M, 52,000 protein in cells grown in the absence or presence of 17β-estradiol. In the cotreatment studies, both concentrations of TCDD decreased the levels of the secreted M, 52,000 protein below the levels observed in the control cells.

### DISCUSSION

TCDD and related halogenated aromatic hydrocarbons elicit a broad spectrum of antiestrogenic responses in the rodent uterus and MCF-7 human breast cancer lines and the results of several studies also indicate that the antiestrogenic activity in a target organ or cell is dependent on functional Ah and estrogen receptors (1). Langdon et al. (11, 12) have reported that the PE01, PE04, and PE06 ovarian adenocarcinoma cell lines are ER positive, and using PE04 cells as a model it was shown that 17β-estradiol (10 or 0.1 nM) induced cell proliferation and the antiestrogen tamoxifen inhibited this induced response (12). The results of studies reported herein clearly demonstrate that after treatment of the ovarian cells with 10 nM [3H]TCDD, the nuclear extracts from these cells contained a specifically bound protein which sedimented at 7.5 to 7.9 S in a sucrose gradient (Fig. 1). Although the levels of the nuclear Ah receptor complex in these cells varied from 23 to 87 fmol/mg protein, the concentration and sedimentation properties of the ovarian cell nuclear Ah receptor complex were similar to those reported for other human cell lines (21–29).

The induction of CYPIA1 gene expression by TCDD has been extensively investigated (30–32) and the results indicate that the nuclear Ah receptor complex acts as a nuclear ligand-responsive transcription factor (6) which interacts with specific genomic sequences (DREs) located in the 5′-flanking region of the CYPIA1 gene (30–32). The induction of CYPIA1 by TCDD and related compounds is a convenient indicator of Ah responsiveness and, in this study, responsiveness to TCDD was determined by the induction of EROD activity, a P450IA1-dependent monoxygenase, and P450IA1 mRNA levels. The results in Tables 1 and 2 show that EROD and P450IA1 mRNA inducibility was observed only in the PE04 cell line and no significant induction responses were noted in the PE01 and PE06 cells. This observation was unexpected but not unique since previous studies have reported that although MDA-MB-231 human breast cancer cells express the Ah receptor which accumulates in cell nuclei after treatment with TCDD, the induction of CYPIA1 gene expression by TCDD is not observed (22). The results of this study show that the

<table>
<thead>
<tr>
<th>Treatment (nM)</th>
<th>Duration (h)</th>
<th>Relative levels of</th>
<th>Control (DMSO)</th>
<th>17β-Estradiol (1)</th>
<th>TCDD (0.1)</th>
<th>TCDD (1.0)</th>
<th>TCDD (1.0) + 17β-estradiol (1.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Secreted M, 52,000 protein compared to MCF-7 cells (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (DMSO)</td>
<td>48</td>
<td>209 ± 30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17β-Estradiol (1)</td>
<td>48</td>
<td>316 ± 41</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCDD (0.1)</td>
<td>48</td>
<td>169 ± 22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCDD (1.0)</td>
<td>48</td>
<td>141 ± 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCDD (1.0) + 17β-estradiol (1.0)</td>
<td>48</td>
<td>164 ± 26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCDD (1.0) + 17β-estradiol (1.0)</td>
<td>48</td>
<td>160 ± 13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* significantly higher (P < 0.01) than levels in MCF-7 cell (10 ± 10%).
* significantly lower (P < 0.01) than in PE04 cells treated with 1 nm 17β-estradiol for 48 h.
PE01 and PE06 ovarian cancer cell lines are similar to the MDA-MB-231 cells in which constitutive and induced CYP1A1 gene expression are repressed. The repressed Ah-responsiveness in the ovarian adenocarcinoma cells may be due to at least two factors, namely, the expression of high levels of nuclear proteins which repress CYP1A1 gene expression or a nonfunctional nuclear Ah receptor. The results of the gel mobility shift assay for nuclear extracts from PE01, PE04, and PE06 cells (Fig. 2) show that an intense retarded band (i.e., a DRE-Ah receptor complex) was formed with the nuclear Ah receptor from the Ah-responsive PE04 cells. This band was present in nuclear extracts from PE06 cells but was not inducible by TCDD whereas only minimal induction of the retarded band was observed for the PE06 cells. Since the interaction of the nuclear Ah receptor complex with genomic DREs is required for TCDD-induced CYP1A1 gene expression, the failure to observe induction in PE01 and PE06 cells is consistent with the results of the gel shift assay (Fig. 2).

The effects of TCDD on the proliferation of the Ah- and ER-responsive PE04 cells was also determined and the results show that TCDD significantly inhibited 17β-estradiol-induced growth (Fig. 3). Comparable growth inhibition of this cell line was previously observed using 1 μM tamoxifen (12). The results showed that although all three cell lines were ER positive, 17β-estradiol stimulated proliferation in the PE04 and PE06 but not the PE01 cells. The results also showed that TCDD did not inhibit 17β-estradiol-stimulated proliferation of PE01 cells but did exhibit antiestrogenicity in the PE06 cells. These results were in contrast to the failure of TCDD to induce CYP1A1 gene expression in this cell line (Tables 1 and 2). Thus, in PE06 cells TCDD appears to be a partial Ah receptor agonist and this observation is not unprecedented since the antiestrogenic responses induced by 6-methyl-1,3,8-trichlorodibenzofuran in MCF-7 human breast cancer cells can be observed without induction of the CYP1A1 gene but with the formation of significant levels of the nuclear Ah receptor complex liganded with 6-methyl-1,3,8-trichlorodibenzofuran (33).

The M, 52,000 protein (procathepsin D), a serine protease, is used clinically for human breast tumors as a negative prognostic indicator for disease-free survival (34, 35). There is evidence from model studies (36) that the M, 52,000 and 34,000 (cathespin D) proteins may play a role in the degradation of extracellular matrix and thereby facilitate tumor invasiveness. The M, 52,000 protein is estrogen inducible in MCF-7 human breast cancer cells (2, 36–38). The results in Table 3 compare the levels of the secreted M, 52,000 protein in MCF-7 and PE04 cells using a SDS-polyacrylamide gel electrophoresis double-staining assay procedure (20). Previous studies in this laboratory have shown that the level of immunoreactive M, 52,000 protein secreted by MCF-7 cells was 305 fmol/mg protein (20) and there was a linear correlation between the radioimmunoassay and double-staining procedures for detecting this protein. The results in Table 2 indicate that the concentration of secreted protein in the untreated PE04 cells was greater than 2-fold higher than observed in MCF-7 cells. The results also showed that 17β-estradiol caused a time-dependent increase and TCDD caused a concentration-dependent decrease in the levels of the M, 52,000 protein secreted by PE04 cells. Moreover, in the cotreatment studies, TCDD significantly inhibited the 17β-estradiol-induced secretion of the M, 52,000 protein in the PE04 cell line. TCDD also inhibited the 17β-estradiol-induced secretion of the M, 52,000 protein in MCF-7 cells (2) whereas the antiestrogen tamoxifen did not exhibit antiestrogenic activity for this response.

The results of this study demonstrate that, like the MCF-7 human breast cancer cell line, PE04 ovarian cancer cells express both the Ah receptor and the ER and TCDD exhibits antiestrogenic activity in these cells. The efficacy of antiestrogen treatment with tamoxifen for ovarian cancer is variable (39–45). It is noteworthy that the signal transduction pathway associated with the Ah receptor represents an alternative process for the induction of antiestrogenic responses and therefore may be useful for the treatment of estrogen-dependent ovarian and mammary tumors. TCDD, the model compound used for these studies, is highly toxic and is a well-known rodent carcinogen (46) and would not be acceptable for clinical studies. However, studies in this laboratory have shown that several relatively nontoxic alkylated polychlorinated dibenzo-furans which are Ah receptor agonists also exhibit antiestrogenic activity and therefore may be useful as potential antiestrogens for future clinical studies (33, 47, 48).

REFERENCES


Characterization of the Aryl Hydrocarbon Receptor and Aryl Hydrocarbon Responsiveness in Human Ovarian Carcinoma Cell Lines

C. Rowlands, V. Krishnan, X. Wang, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/53/8/1802

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.