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Estrogen- and Antiestrogen-responsiveness of HEC1A Endometrial Adenocarcinoma Cells in Culture

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HEC1A endometrial cancer cells express the wild-type form of the estrogen receptor (ER) and 17β-estradiol (E2) induces proliferation of these cells. In contrast, tamoxifen only causes a minimal increase (<20%) in cell proliferation. In HEC1A cells transiently transfected with the C3-Luc plasmid derived from the complement C3 gene, both E2 and tamoxifen exhibited ER agonist activity and tamoxifen was also a partial antagonist for this response. The relative ER agonist/antagonist activities of E2, tamoxifen and ICI 182,780 were also investigated in HEC1A1 cells transiently transfected with two E2-responsive plasmids, pCATHD-CAT and pCKB-CAT which contain 5′-promoter inserts from the cathepsin D and creatine kinase B genes, respectively. The results showed that E2 and tamoxifen induced reporter gene activity in cells transiently transfected with both constructs. ICI 182,780 exhibited partial ER agonist activity only in cells transiently transfected with pCKB-CAT and antagonized E2-induced reporter gene activity using both the CKB- and CATHD-derived constructs. These results demonstrate that HEC1A endometrial cancer cells are E2-responsive and represent a useful cell culture model for understanding hormone/antihormone-induced endometrial cell responses. **© 1998 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

Endometrial cancer is an important malignancy of the female reproductive tract and in 1990, an estimated 33,000 new cases were diagnosed in the United States [1–3]. Development of this disease in women is associated with exposure to unopposed estrogens and progestins have been utilized in hormone-dependent treatment of endometrial cancer [4–11]. Antiestrogens such as tamoxifen and 4′-hydroxytamoxifen which are used in clinical treatment of breast cancer in women exhibit ER agonist activity in the endometrium and in endometrial cancer cells and there is some concern regarding possible increased incidence of endometrial cancer in women undergoing long term tamoxifen therapy [12–20].

Ishikawa endometrial cancer cells are one of the few estrogen-responsive endometrial cancer cell lines which can be utilized as a model for studying the effects of hormones and antihormones. Although 17β-estradiol (E2) and/or tamoxifen stimulate growth and induce expression of several genes and/or related activities in Ishikawa cells [21–28], hormone-induced expression of cathepsin D was not observed in this cell line [29, 30] whereas estrogen-responsiveness of this gene has been extensively characterized in other hormone-responsive tissues and cell lines including MCF-7 human breast cancer cells [30–34]. The reasons for cell-specific differences in cathepsin D gene expression in ER-positive MCF-7 and Ishikawa cells are unknown but may be related to endometrial factors which inhibit estrogen receptor (ER)-mediated transactivation [29]. Research in this laboratory is focused on identifying functional enhancer sequences required for estrogen-induced transactivation of cathepsin D, heat shock protein 27 and other estrogen-responsive genes and studying mechanisms of crosstalk between the ER and aryl hydrocarbon receptor (AhR) signaling pathways [35–38]. Therefore, we have initiated studies on several endometrial cancer cell lines for both estrogen- and Ah-responsiveness and this report describes results obtained with HEC1A endometrial cancer cells which exhibit an estrogen-respon-

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sive phenotype. ERα is expressed in this cell line and E2 induces cell HEC1A1 proliferation. Constructs containing inserts from three E2-responsive genes in the uterus, namely complement C3, cathepsin D and creatine kinase B, were used in transient transfection studies and both E2 and tamoxifen but not ICI 182,780 were ER agonists.

MATERIALS AND METHODS

Chemicals and biochemicals

17β-Estradiol (E2), tamoxifen and benzo[a]pyrene were purchased from Sigma Chemical Co. (St. Louis, MO). The antiestrogen ICI 182,780 was provided by Alan Wakeling (Zeneca Pharmaceuticals, Macclesfield, U.K.). Mouse ER antibody (IgG2a) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). All other chemicals and biochemicals were the highest quality available from commercial sources.

Oligonucleotides and plasmids

The human ER (hER) expression plasmid was provided by Ming Jer-Tsai, Baylor College of Medicine (Houston, TX). The pCKB-CAT construct containing 2.9 kb of the 5′-flanking region from the rat creatine kinase B gene was obtained from Pamela Benfield, Dupont Corp. (Wilmington, DE) [39]. The human cathepsin (pCATH-CAT) construct contains a cathepsin D promoter insert (−365 to −10) ligated into a pBL/TATA/chloramphenicol acetyl transferase (CAT) plasmid derived from pBL/CAT2. The C3-Luc plasmid was obtained from Donald McDonnell, Duke University Medical School (Durham, NC) and contained a 1.8 kb fragment (−1807 too +58) from complement C3 gene promoter linked to a luciferase reporter gene. The wild-type and mutant estrogen responsive elements (ERE) were prepared by the Gene Technologies Laboratory, Texas A&M University and used for gel mobility shift assay as previously described [35].

Cells

HEC1A, MCF-7, KLE and HEC1B cancer cells were obtained from the American Type Culture Collection (Rockville, MD) and maintained in DMEM/F-12 medium containing phenol red and supplemented with 5% fetal bovine serum plus 10 ml/l antibiotic/antimycotic solution (Sigma Chemical Co., St. Louis, MO) in an air/carbon dioxide (95:5) atmosphere at 37°C. For cell proliferation and transient transfection experiments, cells were grown in DMEM/F-12 medium without phenol red and in 1 or 5% fetal bovine serum (stripped with dextran-coated charcoal) 24 h before treatment with chemicals.

Preparation of nuclear extracts

Cells suspensions were treated with 10 nM E2 and incubated for 2 h. Cells were then harvested by centrifugation and washed twice in 20 ml HEGD buffer (25 mM Hepes, 1.5 mM EDA, 10% glycerol, 1.0 mM dithiothreitol, pH 7.6). The washed cell pellet was resuspended in 3 ml of HED buffer (same as HEGD buffer without the glycerol) and incubated for 10 min, centrifuged and resuspended in 1.5 ml HEGD buffer and homogenized using a tight Teflon pestle/drill apparatus. The homogenate was transferred to a centrifuge tube by adding 20 ml HEGD buffer and centrifuged at 1500 *g* for 10 min. The pellet was then resuspended in 3 ml of HEGD buffer containing 0.5 M potassium chloride (pH 8.5), incubated for 1 h at 4°C and then centrifuged at 105,000 × *g* for 35 min at 4°C. The resulting supernatants represented the nuclear extract fraction which was used for gel electrophoretic mobility shift assays. Nuclei prepared by this method were found to be intact and appeared to be greater than 90% free of extranuclear cellular contamination, as determined by microscopic examination and trypan blue staining.

Gel electrophoretic mobility shift assay

9 pmol of synthetic human ERE oligonucleotide was [^{32}P]labeled at the 5′ end using T4-polynucleotide kinase and [γ^{32}P]ATP. For the ER:ERE binding assay, nuclear extracts (10 μg) from the control (DMSO) and 10 nM E2-treated cells were incubated in HEGD buffer with 1 μg poly[d(I-C)] for 10 min at 20°C to bind non-specific DNA-binding proteins. A 100-fold excess of unlabeled wild-type and mutant ERE was added for the competition experiments and incubated at 20°C for 5 min. Following addition of [^{32}P]-labeled DNA, the mixture was incubated for an additional 15 min at 20°C. The reaction mixture was loaded onto a 5% polyacrylamide gel and electrophoresed at 110′V in 0.9 Tris–borate and 2 mM EDTA, pH 8.0. Gels were dried and protein–DNA complexes were visualized by autoradiography.

Western blot analysis

HEC1A and MCF-7 cells were seeded in 150 × 25 mm petri dish at 70% confluence and allowed to grow to 90% confluence. Cells were harvested and centrifuged and the resulting cell pellet washed 3 times with ice-cold phosphate-buffered saline. Cells were lysed in 350 μl of ice-cold lysis buffer [50 mM Tris–HCl, 4 mM EDTA, 150 mM potassium chloride, 2.5 μg/ml each of antipain and leupeptin, 1 μg/μl phenylmethylsulfonyl fluoride, 1% Triton x-100 (pH 7.4)]. Lysate was shaken at 4°C for 10 min, centrifuged at 15,000 × *g* for 30 min and the protein concentration of the clear supernatant was determined. Protein aliquots from HEC1A (100 μg) and MCF-7 (60 μg) lysates were separated on 12%

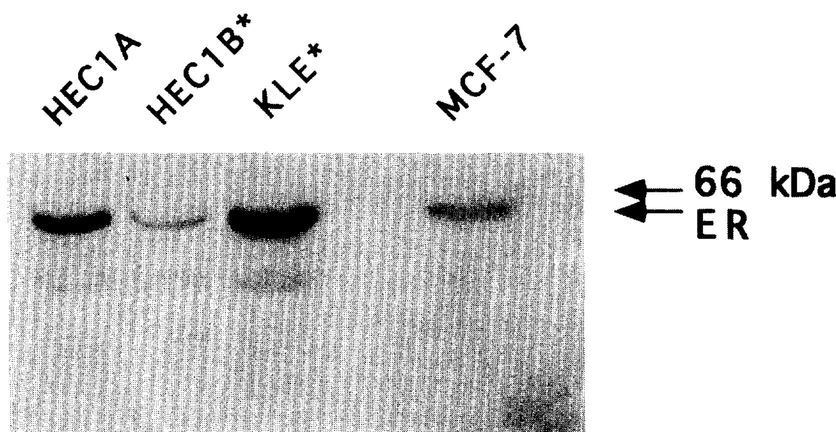

Fig. 1. Detection of immunoreactive ER in HEC1A, HEC1B, KLE and MCF-7 cancer cell lines. Whole cell extracts (100 μg per well) were subjected to SDS-PAGE, followed by transfer to PVDF membrane as described in Section 2. ER was detected using IgG2a (0.1 μg/ml) as a primary antibody and IgG anti-mouse peroxidase conjugate. A 66 kDa immunoreactive band with variable intensity was detected in all cell extracts.

SDS gel and transferred to PVDF membrane. The membrane was blocked for 1 h with 5% milk in PBS (blocking buffer). ER mouse monoclonal IgG2a (0.1 μg/ml) was added to the blocking buffer and incubated for 1 h with gentle shaking. The blot was washed (3×) for 5 min with rinse buffer (0.05% Tween 20 in PBS). IgG antimouse peroxidase conjugate (1:1000 dilution) was added to the rinse buffer and incubated for 1 h. After washing (3×) for 5 min, bound antibodies were detected with an ECL Western Blotting Kit (Amersham Life Science, Arlington Heights, IL). ER antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Cell proliferation

Cells were grown in Dulbecco's essential medium, 10 ml antibiotic–antimycotic solution, 2.2 g sodium bicarbonate and 1% strip bovine serum stripped with dextran-coated charcoal. HEC1A cells (50,000 per well) were seeded in six-well plates and treated with the test compounds; medium was changed every 2 days and test compounds were also added. After 10 days of treatment, cells were harvested and counted using Coulter Z1 cell counter. Results are expressed as means ± SD for 3 separate determination.

Transient transfection and CAT assays

Sub-confluent cells grown in 5% fetal bovine serum stripped with dextran-coated charcoal were transiently transfected with pCKB-CAT or pCATHD-CAT (10 μg/plate) plus hER expression plasmids (5 μg/plate) using the calcium phosphate precipitation method. After 18 h, cells were then treated with DMSO, 10 nM E2, antiestrogens or various combinations for 48 h. Cells were then washed twice with phosphate-buffered saline and scraped from the plates. Cell lysates were prepared in 0.1 ml 0.25 M Tris–HCl, pH 7.8, by three freeze–thaw cycles and sonication (3 min) in a ultrasonic bath. CAT activity was determined using 0.2 mCi D-treo-[dichloroacetyl-1-^{14}C] chloramphenicol (54 Ci/mol, Amersham) and 4 mM acetyl CoA as substrates. The protein concentration was determined using bovine serum albumin as a standard and the same concentration of protein was used for each treatment group. Following thin-layer chromatography, the regions of the plate containing acetylated products were visualized by autoradiography and quantitated using a Betagen Betascope 603 blot analyzer. CAT activity was calculated as the percentage of that observed with DMSO-treated cells alone. The experiments were carried out at least in triplicate unless stated otherwise and results are expressed as means ± SD.

Luciferase assay

Cells were plated on 60 mm petri dishes to 50% confluency in DMEM supplemented with dextran-coated charcoal treated 5% fetal bovine serum without phenol red. Cells were transfected with C3-Luc plasmid (4 μg) and a wild-type hER expression plasmid (1 μg). Cells were treated for 48 h with E2 (10 nM), tamoxifen (10 nM, 1 μM, and 0.1 μM) and DMSO. After incubation, cells were washed twice with PBS and scraped from the plates. Cells were lysed using Reporter Lysis 1× buffer (Promega, Madison, WI) and by one freeze–thaw cycle. Luciferase assays were performed using the Luciferase Assay System with Reporter Lysis Buffer from Promega. The intensity of light emission from assays of cell extracts containing 20 μg of total protein was determined using a Packard 1600 liquid scintillation counter.

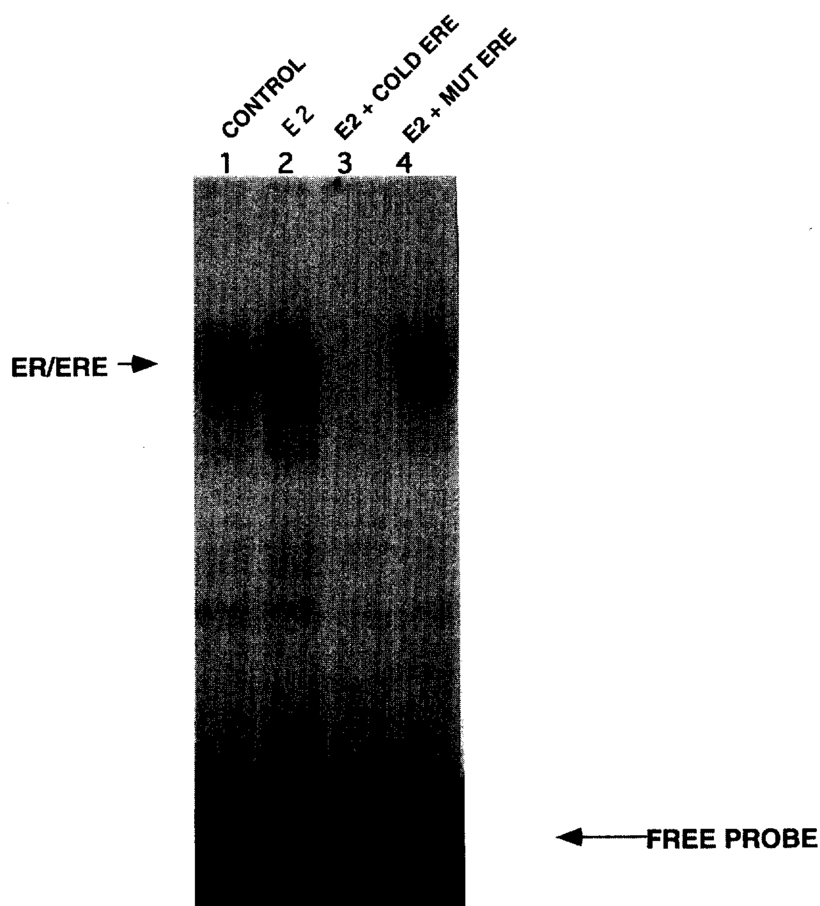

Fig. 2. Gel retardation analysis of the ERE-nuclear extract complexes derived from the HEC1A cell line treated with 10 nM E2 or DMSO. The nuclear extracts were incubated with [^{32}P]-labeled ERE, separated by gel electrophoresis, and visualized by autoradiography as described in Section 2. Nuclear extracts from cells treated with DMSO (lane 1) and 10 nM E2 (lane 2) were incubated with [^{32}P]ERE. Lanes 3 and 4 are nuclear extracts from cells treated with E2 and incubated with 100-fold excess of unlabeled wild-type and mutant ERE, respectively. The relative E2/DMSO ratio of the ERE-bound complex for HEC1A was 1.58. The E2-inducible ER-ERE complex band from HEC1A was reduced in intensity after incubation with a 100-fold excess of unlabeled ERE but no decrease was observed after coincubation with excess unlabeled mutant ERE.

Statistical analysis

Data are reported as mean $\pm$ SD. Statistical significance was determined using ANOVA with Duncan's new multiple range.

RESULTS

The results illustrated in Fig. 1 show that whole cell extracts from HEC1A endometrial cancer cells expressed a 66 kDa protein which immunoreacts with ER antibodies as determined by Western blot analysis. This immunoreactive protein was also identified in ER-positive MCF-7 cells and KLE endometrial cells, whereas only trace levels were detected in the ER-negative HEC1B endometrial cancer cell line. Nuclear extracts from control (DMSO) and cells treated with 10 nM E2 bound to a [^{32}P]ERE to form a retarded protein–DNA band in a gel mobility shift assay (Fig. 2, lanes 1 and 2, respectively). In competition studies, 100-fold excess unlabeled wild-type ERE decreased formation of the retarded band (lane 3), whereas co-incubation with mutant ERE had minimal effects on intensity of the retarded band (lane 4).

The effects of E2, tamoxifen, ICI 182,780 and their combination on proliferation of HEC1A cells was also investigated (Table 1). Treatment with 10 nM E2 resulted in a 68% increase in cell proliferation, whereas tamoxifen alone increased cell growth (11%) only at the 100 nM concentration and 1000 nM tamoxifen decreased cell proliferation. In cells cotreated with 10 nM E2 plus tamoxifen (100 or 1000 nM), hormone-induced proliferation was significantly inhibited. The effects of the 'pure antiestrogen' ICI 182,780 on growth of HEC1A cells in the absence or presence of 10 nM E2 was also determined; 100 and 1000 nM ICI 182,780 alone significantly decreased cell proliferation compared to

Table 1. Effects of E2, tamoxifen and ICI 182,780 and their combination on proliferation of HEC1A cells[a]

Treatment	Concentration (nM)	Cell No. ($\times 10^{-5}$) ($\pm$SD)
DMSO		5.52 ± 0.11
E2	10	9.26 ± 0.73[b]
Tamoxifen	10	5.07 ± 0.55
Tamoxifen	100	6.14 ± 0.45[b]
Tamoxifen	1000	4.29 ± 0.28[d]
Tamoxifen + E2	100 + 10	4.84 ± 0.21[c]
Tamoxifen + E2	10 + 10	3.12 ± 0.17[c]
ICI 182,780	100	3.41 ± 0.32[d]
ICI 182,780	1000	0.36 ± 0.10[d]
ICI 182,780 + E2	100 + 10	4.41 ± 0.91[c]
ICI 182,780 + E2	1000 + 10	0.76 ± 0.05[c]

[a]HEC1A cells were treated with various concentrations of hormone/antihormone for 10 days as described in Section 2 and the number of cells in each treatment group was determined using a Coulter Counter. At least 3 replicates were determined for each treatment group and results are expressed as means $\pm$ SD.
[b]Significantly higher ($p < 0.05$) than observed for E2 alone.
[c]Significantly lower ($p < 0.05$) than observed for E2 alone.
[d] Significantly lower ($p < 0.05$) than observed for controls (DMSO).

DMSO (control)-treated cells; the antiestrogen also significantly inhibited hormone-induced growth (Table 1).

Previous studies have demonstrated that both E2 and tamoxifen induce uterine complement component C3 [40] and induction by tamoxifen is AF-1 dependent [41–43]. Therefore, the effects of E2, tamoxifen and E2 plus tamoxifen on luciferase activity were determined in HEC1A cells transiently transfected with pC3-Luc (Table 2). Both E2 and

Table 2. Luciferase activity in HEC1A cells transiently transfected with C3-Luc and treated with E2, tamoxifen or their combination[a]

Treatment	Concentration (nM)	Relative activity
DMSO	–	100 ± 23
E2	10	628 ± 44[b]
Tamoxifen	10	67 ± 12
Tamoxifen	100	111 ± 9
Tamoxifen	1000	615 ± 217[b]
Tamoxifen + E2	10 + 10	666 ± 173[b]
Tamoxifen + E2	100 + 10	922 ± 24[b]
Tamoxifen + E2	1000 + 10	973 ± 300[b]
ICI 182,780	10	95 ± 9
ICI 182,780	100	134 ± 27
ICI 182,780	1000	169 ± 93
ICI 182,780 + E2	10 + 10	238 ± 17[c]
ICI 182,780 + E2	100 + 10	108 ± 18[c]
ICI 182,780 + E2	1000 + 10	60 ± 19[c]

[a]Cells were transiently transfected with C3-Luc and hER plasmids, treated with hormones/antihormones and luciferase activity determined as described in Section 2. At least 3 replicates were determined for each treatment group and results are expressed as means $\pm$ SD.
[b]Significantly higher ($p < 0.05$) than observed for E2 alone.
[c]Significantly lower ($p < 0.05$) than observed for E2 alone.

1000 nM tamoxifen induced luciferase activity (6.3- and 6.2-fold, respectively) and exhibited ER agonist activity; in cells cotreated with E2 plus tamoxifen, luciferase was higher than that observed for E2 alone. In contrast, ICI 182,780 did not induce luciferase activity but acted as an antiestrogen in cells cotreated with E2 plus ICI 182,780. The results summarized in Fig. 3(A) compare the effects of 10 nM E2, tamoxifen (10, 100 and 1000 nM) and their combination on induction of CAT activity in cells transiently transfected with pCKB-CAT. Both E2 and tamoxifen (all concentrations) significantly induced CAT activity and in cells cotreated with both E2 plus different concentrations of tamoxifen, there was a significant decrease in CAT activity using 100 or 1000 nM tamoxifen compared to cells treated with E2 alone. The antiestrogen ICI 182,780 exhibited partial ER agonist activity and in cells cotreated with E2 plus ICI 182,780, there was a concentration-dependent decrease in E2-induced CAT activity [Fig. 3(B)].

The results illustrated in Fig. 4(A) compare the effects of E2, tamoxifen and their combinations on CAT activity in HEC1A cells transiently transfected pCATHD-CAT. The results show that both E2 (10 nM) and tamoxifen (10 to 1000 nM) caused a 2.67- and 2.19- to 2.53-fold induction of CAT activity, respectively. In cells cotreated with E2 (10 nM) plus tamoxifen (10 to 1000 nM), the hormone-induced activity was not significantly affected at any concentration of tamoxifen. In a second experiment [Fig. 4(B)] using E2, ICI 182,780 and their combination, the latter compound did not induce CAT activity but significantly inhibited the E2-induced response at all concentrations (10, 100 and 1000 nM).

DISCUSSION

Lifetime exposure to estrogens has been established as an important risk factor for both mammary and endometrial cancer [4–11]. ER-positive breast cancer cell lines have been extensively used as models for determining mechanisms of hormone-responsiveness, effects of antiestrogens and antineoplastic agents, development of hormone-independence and drug-resistance [44–46]. Ishikawa human endometrial carcinoma cells have been characterized as one of the few E2-responsive endometrial cell lines which can be utilized to investigate factors which regulate proliferation and differentiation [21–30]. E2 induces proliferation of Ishikawa cells and also increases several other E2-regulated responses including alkaline phosphatase activity, progesterone receptor levels, insulin-like growth factor-1 (IGF-1) expression, IGF-1 receptor binding and transforming growth factor α (TGFα) mRNA levels [21–30, 47, 48]. In contrast, E2 decreased epidermal growth factor (EGF) receptor binding and did not affect cathepsin D gene ex-

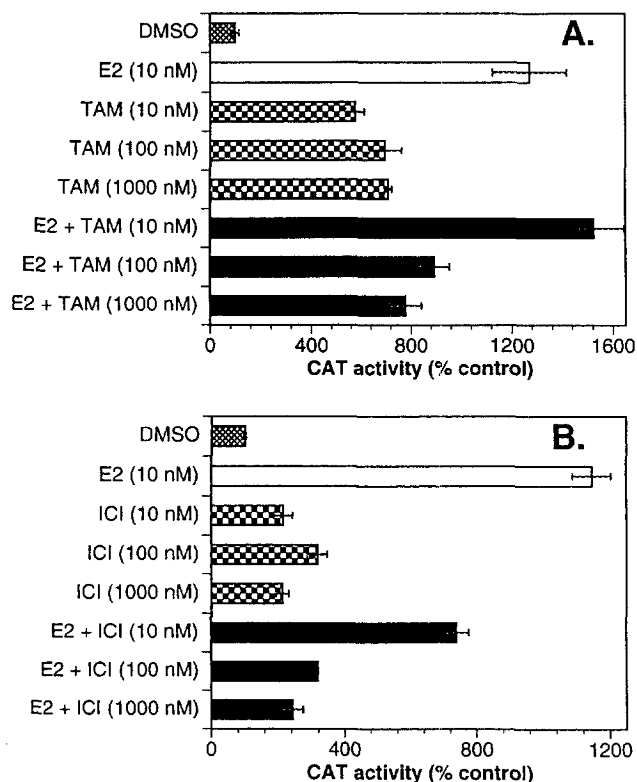

Fig. 3. Effects of E2, tamoxifen, ICI 182,780 and their combination on CAT activity in HEC1A cells transiently transfected with pCKB-CAT. (A) HEC1A cells were co-transiently transfected with hER and pCKB-CAT and treated with DMSO, E2 (10 nM), tamoxifen (10, 100 or 1000 nM), and tamoxifen (10, 100 or 1000 nM) plus E2 (10 nM) as described in Section 2. The relative intensities of the acetylated bands were 100 ± 27, 1273 ± 254, 712 ± 24, 698 ± 115, 558 ± 65, 778 ± 109, 896 ± 102 and 1525 ± 209, respectively. Results are expressed at means ± SD for three separate determinations and compared with CAT activity observed for control (DMSO). E2, tamoxifen and their combinations significantly induced CAT activity ($p < 0.05$) at all concentrations. (B) HEC1A cells were transiently cotransfected with hER and pCKB-CAT and treated with DMSO, E2 (10 nM), ICI 182,780 (10, 100 or 1000 nM) and E2 (10 nM) plus ICI 182,780 (10, 100 or 1000 nM). The relative intensities of the acetylated bands were 100 ± 6, 1147 ± 100, 216 ± 48, 318 ± 51, 213 ± 33, 740 ± 66, 318 ± 28 and 244 ± 52, respectively. Results are expressed as means ± SD for three separate determinations and compared with CAT activity observed for control (DMSO). E2 and ICI 182,780 alone significantly induced CAT activity ($p < 0.05$); ICI 182,780 also significantly inhibited CAT activity induced by E2 at all concentrations.

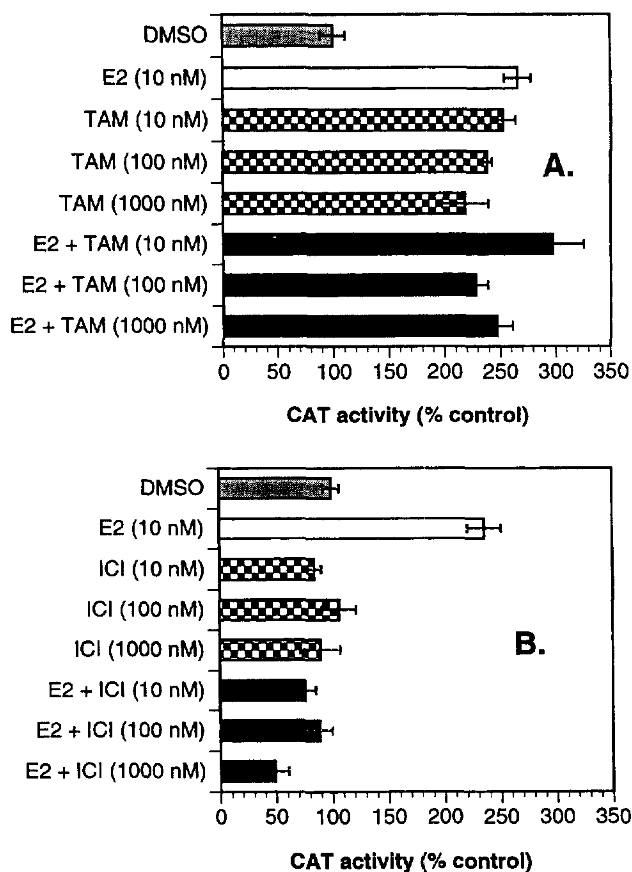

Fig. 4. Effects of E2, tamoxifen, ICI 182,780 and their combinations on CAT activity in HEC1A cells transiently transfected with pCATH-CAT. (A) HEC1A cells were transiently cotransfected with hER and pCATHD-CAT and treated with DMSO, E2 (10 nM), tamoxifen (10, 100 or 1000 nM) and E2 (10 nM) plus tamoxifen (10, 100 or 1000 nM) and CAT activity was determined as described in Section 2. The relative intensities of the acetylated bands were 100 ± 19, 266 ± 20, 253 ± 20, 239 ± 7, 219 ± 36, 298 ± 48, 338 ± 20 and 247 ± 24, respectively. Results are expressed as means ± SD for three separate determinations and CAT activities are expressed relative to DMSO. E2 and tamoxifen significantly induced CAT activity ($p < 0.05$); in all cells cotreated with E2 plus tamoxifen, there was not a significant decreased in E2-induced activity. (B) HEC1A cells were transiently cotransfected with hER and pCATHD-CAT and treated with DMSO, E2 (10 nM), ICI 182,780 (10, 100 or 1000 nM) and E2 (10 nM) plus ICI 182,780 (10, 100 or 1000 nM) and CAT activity was determined as described in Section 2. The relative intensities of the acetylated bands were 100 ± 12, 236 ± 27, 85 ± 10, 107 ± 26, 90 ± 32, 49 ± 21, 89 ± 21 and 76 ± 19, respectively. Results are expressed as means ± SD for three separate determinations and CAT activity was compared to DMSO. E2 significantly induced CAT activity ($p < 0.05$) and ICI 182,780 significantly inhibited CAT activity induced by E2. ICI 182,780 alone did not significantly induce activity.

pression or reporter gene activity in cells transiently transfected with plasmids containing specific 5′-flanking sequences from the cathepsin D gene promoter [29, 30]. The ER agonist/antagonist activities of tamoxifen or its active metabolite 4-hydroxytamoxifen have also been investigated in Ishikawa cells since there has been some concern regarding development of endometrial cancer in women undergoing antiestrogen therapy for treatment of breast cancer [12–20]. Tamoxifen or hydroxytamoxifen induces cell proliferation in some studies [27, 28], enhanced IGF-1 expression, PR and EGF receptor binding but decreased TGFα mRNA levels [27, 28, 47, 48]. Thus, both E2 and tamoxifen or 4-hydroxytamoxifen induced some of the same responses in Ishikawa cells but also showed differences

in activating some prototypical E2-induced responses (e.g. induction of TGFα and EGF receptor binding).

Research in this laboratory has focused on the development of cell culture models for studying the mechanisms of crosstalk between the ER and aryl hydrocarbon receptor (AhR) [35–38] and for development of AhR-based antiestrogens for clinical treatment of breast cancer [49]. Results of preliminary studies suggested that HEC1A endometrial cancer cells might be appropriate for the proposed research; however, reports in the literature on the E2-responsiveness of this cell line were conflicting. For example, in one study, it was reported that E2 activated the migration potential of HEC1A (and Ishikawa) cells through a basement membrane and tamoxifen antagonized the estrogenic response [50]. In contrast, Nguyen and coworkers [51] reported that HEC1A cells were negative for the ER (1.9 fmol/mg). The results illustrated in Fig. 1 demonstrated that HEC1A endometrial cancer cells expressed levels of immunoreactive 66 kDa ER protein similar to that observed in MCF-7 breast cancer cells (note: the KLE endometrial cancer cell line also expressed relatively high levels of immunoreactive ER). Moreover, in gel mobility shift assays, E2 induced formation of a specifically-bound ER–ERE retarded band using [^{32}P]ERE and nuclear extracts from HEC1A cells (Fig. 2). These data indicate that wild-type ER is expressed in HEC1A cells and subsequent studies focused on the comparative activity of E2, tamoxifen and the pure antiestrogen ICI 182,780.

Treatment of HEC1A cells with 10 nM E2 caused a 68% increase in cell growth, whereas 100 nM tamoxifen induced only a small (11%) but significant increase in cell proliferation. Tamoxifen consistently induced HEC1A cell proliferation in several studies (up to 20% increase; data not shown); however, the mitogenic activity was always significantly lower than observed for E2. In cells cotreated with E2 plus tamoxifen or ICI 182,780, the hormone-induced proliferation was significantly decreased over a range of concentrations. Thus, tamoxifen exhibits both ER antagonist and weak agonist activities with respect to proliferation of HEC1A cells and this is not inconsistent with some *in vivo* uterine responses observed in animal models [52].

The complement C3 gene is induced by both estrogens and antiestrogens in the rodent uterus [40] and pC3-Luc, a construct containing the human C3 gene promoter linked to a luciferase reporter, has been extensively utilized as a model for delineating the role of various ligands, activation function (AF) domains of the ER and cellular environment on estrogen/antiestrogen-induced transactivation [41–43]. In HEC1A cells transiently transfected with C3-Luc, both E2 and tamoxifen induced luciferase activity (Table 2) and these data were similar to *in vivo* responses observed in the rodent uterus. In contrast, previous studies showed that tamoxifen did not induce luciferase activity in ER-positive breast cancer cell lines transiently transfected with C3-Luc [43] and the results clearly demonstrate the importance of cell-specific factors which modulate ligand-dependent ER-mediated transactivation.

The effects of E2, tamoxifen and their combination were also determined using two additional E2-responsive constructs in which reporter gene (CAT) activity is regulated by CKB (Fig. 3) and cathepsin D (Fig. 4) gene promoter sequences. E2 induced CAT activity in HEC1A cells transiently transfected with either pCKB-CAT or pCATH-CAT constructs and similar hormone-responsiveness has been observed in MCF-7 cells using the same constructs [53]. Moreover, in parallel studies, tamoxifen also exhibited ER agonist activity in transiently transfected HEC1A cells and only minimal interactions were observed in cells cotreated with tamoxifen plus E2. In contrast, ICI 182,780 exhibited ER antagonist activity in HEC1A cells transiently transfected with pCKB-CAT or pCATH-CAT. Surprisingly, ICI 182,780 alone caused a small but significant increase in CAT activity in cells transiently transfected with CKB-CAT plasmid and this represents one of the first examples of the ER agonist activity of this 'pure antiestrogen'. The promoter regions of the cathepsin D, complement C3 and CKB genes contain multiple *cis*-genomic sequences which are required for ER-mediated transactivation [34, 35, 39, 43]. Results of this study clearly demonstrate that both tamoxifen and E2 induce reporter gene activity in transiently transfected HEC1A cells using C3-Luc, CKB-CAT and CATH-CAT constructs suggesting that for E2 and tamoxifen, ligand-dependent differences in transactivation were minimal.

Kuiper and coworkers [54] recently characterized ERβ, a new form of the ER which exhibits binding affinity for estrogens/antiestrogens similar to that described for ERα [55]. The patterns of tissue and cell-type expression for ERα and ERβ are different [54] and there is evidence of functional differences between these two receptors [56]. Watanabe and coworkers [56] recently reported weak expression of ERα and ERβ in HEC-1 endometrial carcinoma cells (origin not given). In transient transfection studies in HEC-1 cells using an ERE-derived construct and ERα or ERβ expression plasmids, E2, but not tamoxifen or raloxifene, induced reporter gene activity in cells expressing ERα or ERβ [56]. In contrast, results summarized in Table 2 and Figs 3 and 4 clearly demonstrate the ERα agonist activity of tamoxifen using constructs containing more complex promoter elements [34, 35, 39, 43]. These results further demonstrate that ligand-dependent transactivation is dependent on promoter and cellular context [41, 42, 57].

Previous studies in Ishikawa endometrial cancer cells reported that cathepsin D and derived constructs were not inducible by E2 [29, 30], whereas both E2 and tamoxifen induced activity in transiently transfected HEC1A cells (Fig. 5). This suggests that there is differential expression of specific factors required for hormone-induced cathepsin D transactivation in ER-positive HEC1A and Ishikawa endometrial cancer cell lines. Current studies are utilizing HEC1A, Ishikawa and other endometrial cancer line as models to investigate factors responsible for differential hormone/antihormone-induced responses and to determine the mechanisms of crosstalk between the ER and AhR signaling pathways.

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