Differential Effects of Estrogen and Antiestrogen on Transforming Growth Factor Gene Expression in Endometrial Adenocarcinoma Cells

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ABSTRACT

While antiestrogens are useful agents in the treatment of breast cancer, the usefulness of these agents in the treatment of endometrial cancer remains controversial. There is some concern that the currently available antiestrogens may have partial agonist activity in uterine tissue. To better understand the mechanisms by which estrogens and antiestrogens modulate growth of endometrial adenocarcinoma cells, we have compared the effects of 17β estradiol and three antiestrogens, 4-hydroxytamoxifen (OH-TAM), ICI 164384, and LY 117018 on proliferation and transforming growth factor (TGF) mRNA accumulation in two human endometrial adenocarcinoma cell lines. In HEC-50 cells, neither estradiol nor antiestrogens had any effect on cell proliferation or TGF mRNA abundance (P < 0.05) but had no significant effect on TGF-α secretion. TGF-α secretion decreased, whereas OH-TAM increased, epidermal growth factor; FBS, fetal bovine serum; ctFBS, twice charcoal-treated fetal bovine serum. In medium supplemented with 1% ctFBS, estradiol increased cell proliferation and TGF-α mRNA (2.72-fold, P < 0.005) and TGF-α secretion (700 ± 156 versus 250 ± 23 pg/106 cells/24 h, P < 0.05), whereas OH-TAM, which also stimulated cell proliferation, reduced TGF-α mRNA abundance (P < 0.05) but had no significant effect on TGF-α secretion. Under these conditions, ICI 164384 and LY 117018 had no effect on either cell proliferation or TGF-α expression. Estradiol treatment decreased, whereas OH-TAM increased, epidermal growth factor receptors in Ishikawa cells. Both estradiol and the antiestrogens decreased TGF-β1 mRNA abundance when cells were grown in media containing 1% ctFBS. In summary, the response of human endometrial adenocarcinoma cells to estrogen and antiestrogens varied between cell lines and was dependent upon the culture conditions used. In addition, OH-TAM, unlike the other two antiestrogens tested, had growth-stimulating effects on Ishikawa cells.

INTRODUCTION

The mechanisms by which estrogens promote growth of human endometrial adenocarcinoma have not been defined, but by analogy with normal endometrial tissue both direct and indirect mechanisms mediated via autocrine and paracrine growth factors from adjacent stromal cells are likely to be involved. In vitro studies have demonstrated that estrogen has a direct effect on proliferation of human endometrial adenocarcinoma cell lines which contain estrogen receptors (1, 2). Furthermore tamoxifen, an antiestrogen of the triphenylethylen series, has been shown to block the effect of estrogen on the induction of progesterone receptor, alkaline phosphatase, and DNA polymerase activity in endometrial cancer cells (3, 4). Antiestrogens have proven useful in the treatment of breast cancer and have been advocated for advanced endometrial cancer (5, 6); however, there is some concern that in the human, as in the rodent, the currently available antiestrogens may have partial agonist activity in the uterus (7). This activity would limit the usefulness of these agents in endometrial cancer and may explain the excess uterine malignancies when tamoxifen is used as chemoprevention in patients at high risk for breast cancer (8, 9).

Previous studies of the effects of estradiol and tamoxifen on endometrial adenocarcinoma cells have found small but significant growth stimulatory effects of both agents on Ishikawa cells (10). In the present study, we have examined the effects of estradiol, OH-TAM, ICI 164384, and LY 117018 on proliferation and transforming growth factor expression in two human endometrial adenocarcinoma cell lines under different culture conditions. The two human endometrial cancer cell lines which we have examined, Ishikawa and HEC-50 cells, express TGF-α, EGF, and EGF-receptor mRNA (11). Both cell lines have detectable estrogen and progesterone receptor mRNA, although the level of expression of these receptors in HEC-50 cells is very much lower than in Ishikawa cells.

MATERIALS AND METHODS

Materials. Phenol red-free Dulbecco’s modified Eagle’s medium plus F-12 and 17β estradiol were obtained from the Sigma Chemical Co. (St. Louis MO) [3H]dCTP was purchased from DuPont Canada (Lachine, Quebec, Canada). OH-TAM and ICI 164384 were gifts from ICI (Macclesfield, Cheshire, United Kingdom). TGF-α was obtained from Upstate Biotechnology, Inc. (Lake Placid, NY). All other ingredients for cell culture medium were purchased from Gibco Ltd., (Burlington, Ontario, Canada).

Cells and Cell Culture. The original Ishikawa cell line was established by Dr. Iwasaki, Tsukuba, Japan (12). HEC-50 cell line was established by Dr. Kuramoto, Kanagawa-ken, Japan (13). Both cell lines were generously provided by Dr. E. Gurpide, Mount Sinai School of Medicine (New York) The cells were routinely passaged in basal medium, a mixture of Ham’s F-12 and Dulbecco’s modified Eagle’s medium (1:1), 10 mM L-glutamine, 1% antibiotic-antimycotic solution, and supplemented with 5% FBS. In experiments in which the effects of estradiol and antiestrogen were examined under estrogen-depleted culture conditions, medium containing 1% ctFBS was used (1). One week before the experiment, cells were changed from basal medium containing 5% FBS to phenol red-free basal medium containing 1% ctFBS.

Cell Proliferation Study. For cell growth experiments, cells were...
plated at approximately 10^4 cells/30-mm dish in medium containing 5% FBS. Cells were plated at a higher density because of the slower rate of proliferation under estrogen-depleted culture conditions. Approximately 10^5 cells/30-mm dish were plated in medium containing 1% ctFBS. On day 2, the medium was replaced with fresh medium containing varying concentrations of estradiol or antiestrogen. Fresh medium was added every other day and the cells were counted on the seventh day. Cells were counted in duplicate or triplicate using a electronic cell counter. Each experiment was completely replicated on two or more occasions.

RNA Extraction and Northern Blot Analysis. The cells were plated in basal medium. Two days later while the cells were still subconfluent, the medium was replaced with fresh medium. Estradiol or antiestrogen were added directly from a 1000-fold stock solution in ethanol to plates at approximately 10^4 cells/30-mm dish in medium containing 5% FBS. On day 2, the medium was replaced with fresh medium containing varying concentrations of estradiol or antiestrogen. Fresh medium was added every other day and the cells were counted on the seventh day. Cells were counted in duplicate or triplicate using a electronic cell counter. Each experiment was completely replicated on two or more occasions.

RESULTS

The effect of estradiol and the antiestrogens on human endometrial adenocarcinoma cell proliferation is shown in Fig. 1. In estrogen-repleted medium, estradiol had no effect, and OH-TAM, ICI 164384, and LY 117018 inhibited the proliferation of Ishikawa cells (Fig. 1A). In contrast, both estradiol and OH-TAM stimulated proliferation of these cells when grown in estrogen-depleted medium (Fig. 1C). Under the latter culture conditions, ICI 164384 and LY 117018 had no significant effect on cell proliferation. In estrogen-repleted medium, ICI 164384 and LY 117018 had no significant effect on proliferation of HEC-50 cells. Under these conditions, however, both estradiol and OH-TAM caused a slight inhibition of HEC-50 cell proliferation at the very highest concentrations tested (Fig. 1B). Neither estradiol nor the antiestrogens had any significant effect on HEC-50 cell proliferation in estrogen-depleted culture conditions (Fig. 1D).

The levels of TGF-α and β1 mRNAs in Ishikawa cells grown in medium supplemented with 5% FBS were compared with
TGF-α AND -β ESTROGEN AND ANTIESTROGEN REGULATION

the HEC-50 cells (Fig. 2). TGF-α mRNA was more abundant in Ishikawa cells compared to HEC-50 cells, whereas the converse was true for TGF-β mRNA. Both TGF-α and β mRNA levels were decreased in Ishikawa cells grown in 1% ctFBS (Fig. 2).

In estrogen-repleted culture conditions, OH-TAM reduced the abundance of TGF-α mRNA in Ishikawa cells. Six h after exposure to 100 nM OH-TAM, the level of TGF-α mRNA was significantly decreased (Fig. 3A). A further decrease was seen 24–48 h after exposure to OH-TAM. This effect was dose dependent, with as little as 1 nM OH-TAM having a significant effect on TGF-α mRNA levels (Fig. 3B). The effect of OH-TAM on TGF-α mRNA abundance could be reversed by estradiol (Fig. 3C). Similar results were obtained with the two other antiestrogens, although, at the concentration tested, OH-TAM was more potent (Table 1). Under these estrogen-repleted culture conditions, estradiol had no effect on TGF-α mRNA levels but significantly increased TGF-α levels in conditioned medium. In contrast, antiestrogens significantly decreased the amount of TGF-α present in conditioned medium (Table 1). In medium containing 5% FBS serum, neither estradiol nor the antiestrogens had any effect on TGF-α mRNA levels in HEC-50 cells (data not shown).

There were no significant effects of estradiol or antiestrogens on the abundance of TGF-β, mRNA in Ishikawa cells grown in medium containing 5% FBS (Fig. 3; Table 2). Neither OH-TAM nor estradiol had any significant effects on TGF-β, mRNA abundance in HEC-50 cells.

To determine whether the reduced TGF-α expression in Ishikawa cells could be functionally important in the OH-TAM-induced growth inhibition, we examined the effects of exogenous TGF-α. When cells were cultured in basal medium supplemented with 5% FBS, OH-TAM significantly decreased the cell number relative to control ($P < 0.01$), but both estradiol and exogenous TGF-α could partially reverse this growth inhibitory effect (Fig. 4). Exogenous TGF-α alone had no significant effect on Ishikawa cell proliferation under these culture conditions.

TGF-α mRNA levels were increased when Ishikawa cells, cultured in basal medium containing 1% ctFBS, were exposed to estradiol. When cells were incubated with 100 nM estradiol for 24 h, the TGF-α mRNA abundance was increased approximately 3-fold (Table 1, Fig. 5). In contrast to the effect of estradiol, treatment with antiestrogens resulted in a small reduction in TGF-α mRNA levels. In the case of OH-TAM, the

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**Fig. 2.** Comparison of TGF-α and TGF-β, mRNA abundance in Ishikawa and HEC-50 cells. Top, Northern blot hybridized with TGF-α cDNA; bottom, hybridization pattern obtained with the TGF-β, cDNA. Kb, kilobases.

**Fig. 3.** The effect of estradiol and OH-TAM on TGF-α and TGF-β, mRNA abundance in Ishikawa cell grown in estrogen-repleted culture conditions. A, time course of the OH-TAM effect on TGF-α and TGF-β, mRNA abundance; B, dose response of the OH-TAM effect on TGF-α mRNA abundance; C, reversal of the OH-TAM effect with estradiol. A 24-h incubation was used for the experiments shown in B and C. In C, the concentration of estradiol and OH-TAM was $10^{-7}$ M. Top, representative Northern blot hybridized with the TGF-α, TGF-β, and Nb-29 cDNAs. The hybridization signals obtained with the TGF-α cDNA (□ or open columns) and with the TGF-β, cDNA (■ and cross-hatched columns) were quantified by densitometer. Bottom, mean ± SEM for five separate experiments. The data, corrected for gel loading, has been expressed relative to the untreated control cultures, arbitrarily attributed a value of 1. *, $P < 0.01$ for the difference from the untreated control cultures. Kb, kilobases; E2, estradiol.

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**Fig. 3A.**

**Fig. 3B.**

**Fig. 3C.**

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Antiestrogens have been proven to be useful in the treatment of breast cancer and are potentially useful in the treatment of other estrogen-dependent neoplasms such as endometrial cancer. However, the excess of uterine malignancies in patients taking tamoxifen for prevention of recurrent breast cancer (8,9) suggests that this agent may have partial agonist activity in the human endometrial tissue (7). Partial agonist activity would limit the usefulness of these agents in the treatment of endometrial cancer. In this study, we have examined the effect of estradiol and three antiestrogens on proliferation of two human endometrial adenocarcinoma cells. In addition, we have investigated the effects of these agents on TGF-α and TGF-β expression in the two cell lines. We have used two estrogen receptor-positive cell lines and examined the effects of estradiol and antiestrogens under estrogen-repleted and estrogen-depleted culture conditions. The first major finding was that, in Ishikawa cells, OH-TAM, but not the other antiestrogens, stimulated cell proliferation in estrogen-depleted culture conditions. However, unlike estradiol, which also stimulated proliferation, OH-TAM inhibited TGF-α expression.

Under estrogen-repleted conditions, the predominant effect of the antiestrogens in Ishikawa cells was growth inhibition and a reduction in TGF-α expression. The latter was documented at both the mRNA and the protein levels. In contrast to MCF-7 breast cancer cells, in Ishikawa cells, OH-TAM was just as effective, if not more effective, than ICI 164384 in inhibiting cell proliferation (18).

Table 1: Effect of estrogen and antiestrogen on expression of TGF-α, in Ishikawa cells

<table>
<thead>
<tr>
<th>Condition</th>
<th>TGF-α mRNA*</th>
<th>TGF-α protein (ng/10^6 cells/24 h)</th>
<th>EGF receptors*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>1.00 ± 0.04</td>
<td>4.01 ± 0.34</td>
<td>0.86 ± 0.02</td>
</tr>
<tr>
<td>OH-TAM</td>
<td>0.67 ± 0.11</td>
<td>1.16 ± 0.12</td>
<td>1.16 ± 0.12</td>
</tr>
<tr>
<td>LY 117018</td>
<td>0.50 ± 0.08</td>
<td>3.43 ± 0.11</td>
<td>1.16 ± 0.12</td>
</tr>
</tbody>
</table>

* P < 0.005 for the difference from the control group.
° Expressed relative to control which was arbitrarily attributed a value of 1.

Protein concentrations, and under these conditions, OH-TAM was at least as potent as estradiol. These data are consistent with earlier published data obtained with Ishikawa cell (15) and with partial agonist activity of this agent in the rat uterus (19). In the Ishikawa cells, as in the rat uterus, this effect was specific to OH-TAM, since the other antiestrogens tested did not stimulate proliferation under these conditions.

Consistent with previously reported data concerning breast cancer cells (20), TGF-α expression was estrogen responsive in Ishikawa cells. However, OH-TAM did not enhance TGF-α expression despite its obvious growth-stimulating effect in Ishikawa cells grown in estrogen-depleted medium. There are a number of possible conclusions that can be derived from this surprising observation. The increase in TGF-α expression does not appear to be a necessary component of the estrogen-induced proliferative response of Ishikawa cells, even though exogenous TGF-α is able to reverse the growth inhibition seen when Ishikawa cells are treated with antiestrogen under estrogen-repleted culture conditions. Thus, it would appear that OH-TAM is able to activate other genes necessary for proliferation while inhibiting TGF-α expression. There is evidence for EGF/TGF-α-independent estrogen-induced proliferation of human breast cancer cells.

**DISCUSSION**

The use of estrogen-depleted culture conditions, in which OH-TAM stimulated cell proliferation. The effect of the other antiestrogens on TGF-α mRNA abundance in Ishikawa cells (Table 2). Neither estradiol nor OH-TAM had any significant effect on TGF-α or TGF-β mRNA levels in HEC-50 cells under these conditions (data not shown).

![Graph: Effect of estradiol (E2) and TGF-α on the antiproliferative action of OH-TAM](https://example.com/graph.png)

**Table 2: Effect of estrogen and antiestrogen on expression of TGF-β, in Ishikawa cells**

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<th>Condition</th>
<th>TGF-β mRNA abundance*</th>
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<tr>
<td>Estradiol</td>
<td>1.23 ± 0.10</td>
</tr>
<tr>
<td>OH-TAM</td>
<td>0.46 ± 0.13</td>
</tr>
<tr>
<td>LY 117018</td>
<td>0.50 ± 0.08</td>
</tr>
<tr>
<td>ICI 164384</td>
<td>0.50 ± 0.08</td>
</tr>
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° Expressed relative to control which was arbitrarily attributed a value of 1.

No effect was observed even when cells were cultured under estrogen-depleted culture conditions. Estrogen receptor mRNA is easily detectable in HEC-50 cells, although the abundance is approximately one-fifth of that seen in Ishikawa cells. Furthermore, HEC-50 cells demonstrate estrogen responsiveness when grown as xenografts in nude mice. The second major finding was that, in Ishikawa cells, OH-TAM, but not the other antiestrogens, stimulated cell proliferation in estrogen-depleted culture conditions. However, unlike estradiol, which also stimulated proliferation, OH-TAM inhibited TGF-α expression.

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![Graph: Effect of estradiol (E2) and TGF-α on the antiproliferative action of OH-TAM](https://example.com/graph.png)

**Fig. 4.** The effect of estradiol (E2) and TGF-α on the antiproliferative action of OH-TAM. Ishikawa cells were grown in basal medium supplemented with 5% FBS. The effects of 100 nM OH-TAM, 100 nM estradiol, and 20 nM TGF-α on cell number are shown. Columns, mean cell numbers ± SEM (bars) of four separate experiments. *P < 0.01 for the difference from the untreated control cultures.**

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is of interest that both estradiol and two of the three antiestrogens tested significantly reduced TGF-β1 mRNA levels in Ishikawa cells under estrogen-depleted conditions. Since TGF-β1 is growth inhibitory for Ishikawa cells (11), this reduction in TGF-β1 expression would favor cell proliferation. However, ICI 164384, which reduced TGF-β1 mRNA abundance to the same extent as OH-TAM, had no growth stimulatory effect.

In conclusion, we have demonstrated that OH-TAM, but not LY 117018 or ICI 164384, can have growth stimulatory effects on human endometrial adenocarcinoma cell lines. However, the growth stimulatory effects of estradiol and OH-TAM appear to be qualitatively different in terms of the expression of the transforming growth factor genes. Our observations also suggest that the TGFs may have little functional role in the estrogen-induced proliferation of uterine adenocarcinoma cells.

ACKNOWLEDGMENTS

The authors thank Dr. Erlio Gurpide for helpful discussions and for providing the Ishikawa cell line.

REFERENCES


Fig. 5. The effect of estrogen and antiestrogen on transforming growth factors mRNA accumulation in Ishikawa cells. Cells were grown in basal medium containing 1% FBS with estradiol (E2) or antiestrogens as indicated at a final concentration of 100 nM for 24 h. The hybridization patterns obtained with the TGF-α, TGF-β1, and Nb-29 cDNAs are shown in a representative autoradiogram. Kb, kilobases.


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