The Effects of Estrogens and Antiestrogens on Hormone-responsive Human Breast Cancer in Long-Term Tissue Culture

Marc Lippman, Gail Bolan, and Karen Huff

SUMMARY

We have established or characterized six lines of human breast cancer maintained in long-term tissue culture for at least 1 year and have examined these lines for estrogen responsiveness. One of these cell lines, MCF-7, shows marked stimulation of macromolecular synthesis and cell division with physiological concentrations of estradiol. Antiestrogens are strongly inhibitory, and at concentrations greater than $3 \times 10^{-7}$ M they kill cells. Antiestrogen effects are prevented by simultaneous treatment with estradiol or reversed by addition of estradiol to cells incubated in antiestrogen. Responsive cell lines contain high-affinity specific estradiol receptors. Antiestrogens compete with estradiol for these receptors but have a lower apparent affinity for the receptor than estrogens. Stimulation of cells by estrogens is biphasic, with inhibition and cell death at concentrations of $17\beta$-estradiol or diethylstilbestrol exceeding $10^{-7}$ M. Killing by high concentrations of estrogen is probably a nonspecific effect in that we observe this response with $17\alpha$-estradiol at equivalent concentrations and in the otherwise unresponsive cells that contain no estrogen receptor sites.

INTRODUCTION

The mechanism by which estrogen stimulates the proliferation of certain target tissues including some breast carcinomas is incompletely understood (16, 17). Part of the difficulty has resulted from the lack of a suitable in vitro model system in which the process could be studied in a monoclonal, completely viable cell population in hormonally defined medium. Many previous studies have examined the effects of estrogens on short-term tumor explants (2-4, 8, 11, 22, 26). All have suffered from one or more of the following problems: experiments performed with a mixed and/or dying cell population; the use of medium with serum containing physiological levels of 1 or more hormones; and the use of pharmacological concentrations of hormones in excess of $10^{-8}$ M to induce effects.

We now report on 6 tissue culture lines of human breast cancer, 2 of which show responsiveness to estrogen. The cells, which have been maintained for at least 1 year in continuous culture, show marked stimulation of macromolecular synthesis and growth when incubated in medium containing physiological concentrations of estradiol.

MATERIALS AND METHODS

Cells and Tissue Culture. Two cell lines, Evsa T and Evsa E, were established from malignant ascitic effusions from a female patient with metastatic breast carcinoma by using minor modifications of previously published techniques (6, 12, 21). Both cell lines were epithelial and morphologically appeared to be non-contact inhibited. Chromosomal analysis of cells after 6 months in culture revealed heteroploidy with a median chromosomal number close to that of fresh malignant material obtained from the same patient (84 versus 76). In addition, characteristic large telocentric marker chromosomes were found in both the tissue culture line and material obtained from paracentesis. Both Evsa T and Evsa E synthesize $\alpha$-lactalbumin as determined by a specific enzymatic assay. MCF-7, a cloned cell line derived from a malignant pleural effusion in a female patient with metastatic breast cancer, was generously provided by Dr. Marvin Rich, Michigan Cancer Foundation, Detroit, Mich. (23). MDA-231, HT-39, and G-11, 3 other human breast cell lines, were provided by Dr. Ronald Herberman, National Cancer Institute, Bethesda, Md., and Dr. Grace Cannon, Litton Biophenetics, Bethesda, Md. The latter 4 lines were also shown to synthesize $\alpha$-lactalbumin (M. E. Lippman, and B. V. Vondanhaar, unpublished observation; the method used to determine $\alpha$-lactalbumin activity enzymatically involves incubation of cell homogenates with $[^{14}C]$UDP-galactose and measures the production of $[^{14}C]$lactose). All cells were grown in monolayer cultures in MEM supplemented with 2x glutamine and 10% fetal calf serum (North American Biologicals Inc., Miami, Fla.)

Preparation of Estrogen-depleted Sera. Physiological concentrations of steroid hormones are present in the serum-containing media in which the cells are usually grown (10). Estradiol concentrations as high as $10^{-8}$ M have been found in some fetal calf sera (10). Therefore, serum was stripped of endogenous steroid by mixing with dextran-coated charcoal twice at 50° for 45 min (1). Removal of
estriol was monitored by adding a trace amount of tritiated estriol at the beginning of the procedure and following the decrease in radioactivity to less than 1% after successive charcoal treatments. Estriol remaining in the undiluted serum was less than 10^{-12} M. The charcoal was removed by centrifugation, and the serum was stored frozen at -20°C until use and sterilized by filtration.

**Steroids.** 17β-[3H]Estriol (100 Ci/mmoles; Amersham-Searle Corp., Evanston, Ill.) was evaporated to dryness, dissolved to ethanol, and stored at -20°C until use. Nonradioactive estriol and diethylstilbestrol solutions were prepared and stored similarly. Tamoxifen (ICI 46474; trans isomer of 1-[(2-dimethylaminothoxy)phenyl]-1,2-diphenylbut-1-ene citrate) and Parke-Davis CI 628, triphenylathylen derivatives with antiestrogenic properties, were similarly prepared.

**Estriol Receptor Assays.** Receptor assays were performed using sucrose density gradients (15), dextrancoated charcoal (20), or protamine sulfate precipitation (7) as noted. Cells prepared for binding studies were grown as noted above but incubated in serum-free medium overnight before harvesting in order to deplete estrogen receptors of endogenously bound steroid. Multiple assays were used in order to substantiate observations of the lack of estrogen receptor in some cell lines. All 3 assay techniques gave results in agreement with each other. One confluent 75-cm culture dish yields 1 ml of cytoplasmic extract at a protein concentration of about 1 mg/ml.

**Precursor Incorporation.** Cells growing in log phase were suspended using trypsin-EDTA and plated replicately in plastic dishes in MEM supplemented with 1% steroid-free serum. After 24 hr the medium was exchanged for fresh MEM with 1% steroid-free serum, and hormones were added in ethanol (final concentration of ethanol was always less than 0.1%). In the experiment described in Table 1, serum-free medium was used instead of medium containing charcoal-treated serum after 24 hr. After 36 hr radioactive leucine, uridine, or thymidine (Amersham Searle) diluted in MEM was added to each dish 1 or 2 hr before the cells were harvested. Each dish usually contained 0.5 µCi of tritium or 0.25 µCi of 14C-labeled precursor; occasionally, less isotope was used. Precursor incorporation is linear for all isotope concentrations used at these time intervals. Cells were harvested by washing the dishes once with ice-cold Dulbecco’s phosphate-buffered saline (pH 7.4), suspending the cells in trypsin-EDTA, and collecting cell pellets by centrifugation. Cell pellets were suspended in ice water and sonically dispersed for 3 sec in a Branson sonicator at the lowest setting. Aliquots were then used for the determination of protein using the method of Lowry et al. (18) or for precipitation in 10% trichloracetic acid. Acid-insoluble counts were collected and washed on 0.45-µm Millipore filters. After drying, the filters were solubilized in Aquasol (New England Nuclear, Boston, Mass.) and counted in a Packard liquid scintillation counter (efficiency for tritium, ~35%; efficiency for 14C ~65%).

**Cell Growth Experiments.** Cells growing in log phase were suspended with trypsin-EDTA and plated in triplicate or quadruplicate in 60-mm Petri dishes at a density of ~50,000 cells/dish. Hormones were added in ethanol (final concentration of ethanol, <0.1%), and at various times cells were collected by suspension in trypsin-EDTA and counted in a hemocytometer.

**RESULTS**

MCF-7 cells show marked stimulation of incorporation of precursors into macromolecules by estriol. In Table 1 the effects of various hormonal additions on [3H]uridine incorporation into RNA are shown. There is a 2-fold increase in uridine incorporation seen in cells incubated in 10^{-8} M estradiol for 48 hr before pulsing with labeled nucleoside (see Chart 5 for dose-response curve for thymidine incorporation). If, on the other hand, the cells are incubated in 10^{-7} M tamoxifen, an antiestrogen, uridine incorporation is about 66% of control values and 30% of estrogen-stimulated values. If the cells are incubated in 10^{-7} M tamoxifen plus 10^{-8} M estradiol, the inhibition by the antiestrogen is partially overcome and uridine incorporation is 93% that seen in cells stimulated with estradiol alone. This experiment was performed under entirely serum-free conditions. Most of the studies that follow were performed using the 1% charcoal-treated serum described in “Materials and Methods.” Comparable results are obtained either way. Similar effects of estrogen and antiestrogen are observed when other precursors such as thymidine are used as also shown in Table 1.

Effects of 10^{-8} M estradiol and 10^{-7} M tamoxifen on cell growth are shown in Chart 1. As shown, physiological concentrations of estradiol clearly stimulate cell division while the antiestrogen tamoxifen is strongly inhibitory. Cell division is stimulated somewhat less markedly than is thymidine incorporation. This may be due to hormonal effects on thymidine pools or transport that exaggerate the effects on thymidine incorporation. Alternatively, in the hormone-stripped media, other factors such as insulin and other growth-promoting peptides may be deficient which may tend to limit the rate of cell division.

The kinetics of the response of these cells to estrogen and antiestrogen is shown in Chart 2. In this experiment, estradiol (10^{-8} M) or tamoxifen (10^{-7} M) is added to cells at time 0. There are no apparent differences between estrogen and control populations of cells for the 1st 14 hr in the experiment shown, after which estradiol-stimulated cells begin to incorporate thymidine at a rate greater than did control cells. Tamoxifen-treated cells show inhibition also beginning at 14 hr. At 36 hr estradiol sufficient to raise the final

<table>
<thead>
<tr>
<th>Addition</th>
<th>[3H]Uridine incorporation (dpm x 10^{-3}/µg protein/hr)</th>
<th>[14C]Thymidine incorporation (dpm x 10^{-3}/µg protein/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.8 ± 0.5*</td>
<td>15.1 ± 0.3</td>
</tr>
<tr>
<td>10^{-8} M estradiol</td>
<td>15.2 ± 0.6</td>
<td>47.4 ± 0.6</td>
</tr>
<tr>
<td>10^{-7} M tamoxifen</td>
<td>4.6 ± 0.3</td>
<td>10.2 ± 0.5</td>
</tr>
<tr>
<td>10^{-8} M estradiol + 10^{-7} M tamoxifen</td>
<td>14.1 ± 0.8</td>
<td>42.3 ± 0.9</td>
</tr>
</tbody>
</table>

* All values are means of quadruplicate determinations ± S.D.
concentration of estradiol to $10^{-8}$ M is added to one-half of the cells incubated in $10^{-7}$ M tamoxifen. Beginning 12 hr later, a sharp rise in nucleoside precursor incorporation is seen in these "rescued" cells such that thymidine incorporation rises to levels exceeding control levels. This 12- to 14-hr lag between steroid addition and phenotypic response is characteristic of some steroid-cell interactions in other systems such as prostate (16). The slow rise in rates of incorporation per unit time in control cells may have many possible explanations. Rates of incorporation may vary as a function of cell density, time from previous plating or manipulation, or production by the cells of various growth-promoting substances. Such possibilities are under investigation. The falloff or plateau in rates of incorporation probably represents some depletion of the medium.

We have observed this rescue phenomenon in 4 separate experiments. Invariably, precursor incorporation rises well above unstimulated levels before falling towards control levels in the rescued cells. This reversibility of tamoxifen inhibition by estradiol, combined with the observation that tamoxifen effects are about 75% prevented by simultaneous estradiol administration, strongly suggests that tamoxifen inhibits the cells via an estrogen-mediated mechanism and not because of a nonspecific toxic effect of the antiestrogen. Furthermore, we believe that the marked rise in thymidine incorporation to levels exceeding control levels in the early period of estradiol rescue of tamoxifen inhibition suggests that the cells may be arrested by antiestrogen in a uniform stage of the cell cycle. When estradiol addition reverses the tamoxifen inhibition, a larger proportion of the cells enter the DNA-synthetic phase of the cell cycle and thymidine incorporation exceeds control levels.

If the cells are allowed to remain in higher concentrations of tamoxifen ($10^{-6}$ M), after about 48 hr the cells round up, detach from the bottom of the dish, and die. Rarely, a few cells per dish may survive and slowly form colonies in the presence of tamoxifen. Tamoxifen-resistant cells appear to occur with a frequency of about $10^{-5}$ to $10^{-6}$. Resistant cells do not differ morphologically from the parental cells but appear to grow very slowly in antiestrogen.

Since these cells are stimulated by estrogen, one would expect them to have estradiol receptor molecules in their cytoplasm. Chart 3 shows sucrose density gradients of cytoplasmic extracts prepared from MCF-7 cells. A peak of $[^3\text{H}]$estradiol binding that sediments at about 8 S is apparent. This peak of binding is almost completely obliterated if the cytoplasmic extracts are incubated with $[^3\text{H}]$estradiol plus either a 100-fold excess of unlabeled estradiol or a 1000-fold excess of tamoxifen. If sucrose density gradients are done in 0.4 M KCl (Chart 4), the peak of $[^3\text{H}]$estradiol binding shifts to about 4 S. This is characteristic of estradiol receptors studied in other systems (15). This peak of binding is completely competed by an excess of unlabeled steroid.

If estradiol is stimulating the cells via a mechanism involving an initial interaction of estrogen with estradiol receptor, one might expect that there would be some correlation between concentrations of estradiol that bind to receptor and concentrations of estradiol that stimulate macromolecular synthesis. In Chart 5 the binding of $[^3\text{H}]$estradiol to receptor using a dextran-coated charcoal assay is plotted along with the dose-response curve of the cells to estradiol. The dissociation constant for the estradiol receptor obtained by Scatchard analysis of the binding data is calculated to be about $6.8 \times 10^{-10}$ M, a value in agreement with an approximate value calculated from previously published data (5). Similar results were obtained using the protamine sulfate technique. We estimate there to be about 60 fmoles of estradiol bound per mg of cytoplasmic protein. The binding data are presented as fmoles of $[^3\text{H}]$estradiol specifically
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bound per ml of cytoplasmic extract as increasing concentrations of unlabeled estradiol are added. In Chart 5 the dose-response curve of estradiol-mediated stimulation of nucleoside incorporation is calculated on a per mg of cell protein basis. The sensitivity of these cells to estradiol is readily appreciated. Concentrations of estradiol exceeding $5 \times 10^{-10}$ M induce as well as $10^{-8}$ M. Thus, in view of the physiological concentrations of estradiol ($\sim 10^{-9}$ to $10^{-8}$ M) in untreated fetal calf serum (10), it is not surprising that some previous studies may have failed to detect estrogen dependency in *in vitro* systems. The dose-response curve of estrogen stimulation is shifted about 10-fold to the left of the binding curve of estradiol to receptor. Thus, concentrations of estradiol sufficient to saturate only about 10 to 15% of receptor sites appear sufficient for maximal induction of the cells. This may be an artifact of the receptor assay methodology in which binding affinities are done *in vitro* on cytoplasmic extracts at 0° and cell induction experiments are done on whole cells at 37°. Differences in rates of incorporation between Charts 2 and 5 represent differences in specific activity of the isotope used.

One mechanism by which antiestrogens are thought to exert their effects is through competition with estrogen for estradiol-binding sites. One would predict from this hypothesis that there would be good agreement between concentrations of tamoxifen that inhibit binding of [$^3$H]estradiol to receptor and concentrations which inhibit macromolecular synthesis. Results are shown in Chart 6. With the use of a dextran-coated charcoal assay, unlabeled tamoxifen totally competes with [$^3$H]estradiol for the receptor, but the apparent affinity for the receptor is about a 1000-fold less than estradiol. Tamoxifen inhibition is not manifested until molar...
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Tamoxifen Concentration (M)
1 7β-Estradiol-3-methyl ether gives the same results as does 17α-estradiol. This suggests that inhibition by high molar concentrations of steroid may be a nonspecific effect not mediated by receptor interaction in our cells in culture. Obviously, such high concentrations of estrogen in vivo are more specific in their effects.

Further evidence as to the nonspecificity of the response of human breast cancer cell lines to high concentrations of estradiol is shown in Chart 8. The effects of estradiol and tamoxifen on 3 other human breast cancer cell lines all of which synthesize α-lactalbumin are shown. These responses may be compared with that for a hormone-responsive cell line, MCF-7, also shown in Chart 8. Physiological concentrations of estradiol (10⁻⁸ M) and tamoxifen at 10⁻⁶ M have no significant effect on macromolecular synthesis in concentrations about 1000 times in excess of those estradiol concentrations that stimulate the cells are achieved (compare with Chart 5). As shown, tamoxifen inhibition of estradiol binding is displaced 2- to 3-fold to the right of the tamoxifen inhibition of nucleoside incorporation curve; i.e., a concentration of tamoxifen that apparently displaces about 10% of [³H]estradiol from receptor sites is sufficient to inhibit macromolecular synthesis by more than 50%. Again, this difference between binding and cell inhibition may be a manifestation of altered binding affinities when measured on cytosols at 10°. Whatever the exact mechanism of tamoxifen inhibition is, it must involve interference with estradiol action at some point since estradiol can reverse the inhibitory effects of tamoxifen (Table 1; Chart 2). Another antiestrogen, Parke Davis Cl 628, induces similar inhibition at identical molar concentrations.

A puzzling aspect of the hormonal management of breast cancer is the fact that many patients show tumor regressions following the administration of pharmacological doses of estrogen (24). As shown in Chart 7, estradiol stimulation of hormone-responsive cells is biphasic; i.e., as 17β-estradiol concentration increases above about 10⁻⁷ M, less stimulation of nucleoside incorporation is seen. At concentrations greater than 10⁻⁸ M, the cells are inhibited below control levels and after about 72 hr most cells will round up, detach from the bottom of the dish, and die. Thus, high molar concentrations of 17β-estradiol can strongly inhibit some human breast cancer cell lines in tissue culture without requiring alterations in other potentially trophic hormones, since these are not variables in our in vitro experimental procedure. This biphasic response is also observed with diethylstilbestrol at similar concentrations (data not shown). The results of substituting the biologically less active 17α-estradiol for 17β-estradiol are seen in Chart 7. 17α-Estradiol does not stimulate thymidine incorporation in the cells under our assay conditions, but there is inhibition of nucleoside incorporation at molar concentrations identi-
the MDA-231, G-11, and HT-39 breast cell lines. Estradiol at 10^{-8} \text{ M} does not stimulate thymidine incorporation in Evsa T or Evsa E but does induce accumulation of \( \alpha \)-lactalbumin. Estradiol at 10^{-8} \text{ M}, which strongly inhibits the hormone-dependent MCF-7 cell lines, also inhibits the 3 hormone-independent cell lines as well as Evsa T and Evsa E. This raises the possibility that inhibitory effects of high concentrations of estradiol are due to some nonspecific toxicity of the steroid at these levels. Data correlating the presence of estrogen receptor in human breast cancer tissue samples with response to endocrine manipulation have strongly suggested that the presence of estrogen receptor is associated with a high likelihood of response to additive estrogen therapy as well as castration, whereas patients lacking receptor very rarely respond to additive therapy (19). This implies that the inhibitory effects of pharmacological concentrations of estrogen may be mediated by a mechanism involving estrogen receptor. Therefore, the G-11, MDA-231, and HT-39 cell lines were examined for the presence of cytoplasmic estradiol receptor. Results are shown in Table 2 using a protamine sulfate assay. As shown, these cell lines have low estrogen receptor activity. We conclude that in our in vitro system high concentrations of estradiol are lethal nonspecifically, since the effect may be observed after treatment with 17\( \alpha \)-estradiol and 17\( \beta \)-estradiol-3-methyl ether and may be seen in receptor-negative, estrogen-unstimulated cell lines.

**DISCUSSION**

In this study we have presented data characterizing the response of several human breast carcinomas in continuous culture to estrogen and antiestrogen treatment. Several features of these systems are noteworthy.

First, as shown in Charts 5 and 7 the MCF-7 cell line is exquisitely sensitive to estradiol. The cells show reproducible stimulation at concentrations greater than 5 \times 10^{-11} \text{ M} estradiol. This suggests that failure to observe estrogen responsiveness in the past may be due in part to inadequate removal of endogenous steroid from the incubation medium, since as little as 5 \times 10^{-10} \text{ M} estradiol may maximally stimulate the cells. This has important clinical ramifications in that it implies that at least some patients who fail to respond to castration therapy may do so because peripheral conversion of adrenal androgen precursors to estrogen (13) may occur at a rate sufficient to maintain circulating estrogen levels above the extremely low concentrations necessary to stimulate the cells.

Another point of interest is the observation that the antiestrogen tamoxifen is capable of inhibiting the MCF-7 cells well below control levels. The inhibition of macromolecular synthesis below control levels is probably not due to a nonspecific toxic effect of the antiestrogen since, as shown in Chart 2 and Table 1, simultaneous administration of tamoxifen with 10-fold less estradiol essentially blocks the inhibition by tamoxifen, and sequential administration of estradiol after 36 hr of tamoxifen inhibition reverses the effect. Also, cell lines with no estradiol receptor activity are unaffected by tamoxifen. Furthermore, this inhibition below control levels is unlikely to be due to interference with residual amounts of estrogen persisting in our "stripped" serum, since these experiments may be reproduced under entirely serum-free conditions where there is no possibility of exogenously added estrogen (see Table 1). Some human breast carcinomas are capable of a variety of steroid bio-transformations (24). It is possible that tamoxifen might interfere with the action of estrogen if it were synthesized \textit{de novo} by these cells in culture. We are currently investigating steroid biosynthesis in these cells. Possibly, estradiol further stimulates production of some essential mRNA species that is necessary for cell proliferation and is normally synthesized. If antiestrogens block even basal elaboration of these essential mRNA's, the cells may die. Although it has generally been maintained that antiestrogens act by competing with estradiol for receptor binding and although this is easily demonstrable as shown in Charts 3 and 6, recent work has suggested that their mechanism of action may be more complex with nuclear binding of (antiestrogen)-(estrogen receptor) complexes (9, 14). For example, as shown in Chart 6, concentrations of tamoxifen sufficient to displace only 10% of \([\text{H}]\)estradiol from receptor consistently block macromolecular synthesis by more than 50%. Tamoxifen could bind to estrogen receptor, translocate to the nucleus, and reduce key mRNA synthesis below control levels by binding to and in some way blocking some important segment(s) of the genome.

Finally, some attention should be devoted to the perplexing issue of the mechanism of inhibition of human breast cancer by high concentrations of estradiol. The sole argument favoring the view that this effect is mediated by estrogen receptor is supplied by the data that show that patients lacking estradiol receptor almost universally fail to respond to the pharmacological administration of estradiol, whereas as many as two-thirds of the estrogen receptor-positive patients have objective responses to additive hormonal therapy (19). On the other hand, steroid concentrations greater than 10^{-6} \text{ M} may show many nonspecific effects on nontarget tissues (25), which probably do not require receptor interactions. In addition, the ability of high concentrations of estradiol to inhibit cells unaffected by tamoxifen (Chart 8) is another point against the specificity of the response. Finally, the weak agonist 17\( \alpha \)-estradiol inhibits the cells at high concentrations as effectively as does 17\( \beta \)-estradiol. Further investigation of this point may help in management of patients with breast cancer.

We hope that the estrogen-responsive human tissue culture system described will be of value in studying the mechanism of action of estradiol. We are currently preparing

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Quantity of estradiol receptor in various human breast cancer cell lines as determined by protamine sulfate assay (\text{fmol/mg prot})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell line</td>
<td>Estrogen receptor \text{ (fmol/mg protein)}</td>
</tr>
<tr>
<td>MCF-7</td>
<td>71.6 ± 12.9</td>
</tr>
<tr>
<td>MDA-231</td>
<td>6.6</td>
</tr>
<tr>
<td>HT-39</td>
<td>4.2</td>
</tr>
<tr>
<td>G-11</td>
<td>5.0</td>
</tr>
</tbody>
</table>
variant cell lines resistant to either antiestrogen or high concentrations of estradiol to provide contrasting cell lines for study.

ACKNOWLEDGMENTS

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We thank Dr. J. Whang-Peng for performing the chromosomal studies. We also thank Dr. Marvin Rich of the Michigan Cancer Foundation for generously providing us with the MCF-7 cell line, and we thank Dr. Ronald Herberman, National Cancer Institute, for the G-11, HT-39, and MDA-231 cell lines.

REFERENCES


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