Characterization and Quantitation of Antiestrogen Binding Sites in Estrogen Receptor-positive and -negative Human Breast Cancer Cell Lines

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ABSTRACT

Antiestrogens are useful in the treatment of endocrine-responsive breast cancers in humans. In an attempt to understand the mechanisms underlying their estrogen antagonism and antitumor character, we have examined the interaction of antiestrogens with three human breast cancer cell lines that differ markedly in their estrogen receptor content and in their sensitivity to growth suppression by antiestrogens. MCF-7 cells have high levels of estrogen receptor, and their growth is inhibited markedly by antiestrogens; T47D cells contain low levels of estrogen receptor, and their growth is suppressed weakly by antiestrogens; and MDA-MB-231 cells contain no detectable estrogen receptors, and their growth is unaffected by antiestrogens. In addition to binding to the estrogen receptor, antiestrogens are found to be associated with binding sites that are distinct from the estrogen receptor. These estrogen-noncompetible but antiestrogen-competible binding sites are present in the 800 and 12,000 × g supernatants of all three breast cancer cells. The antiestrogen binding sites are pelleted upon centrifugation at 100,000 or 180,000 × g and appear to be associated with microsomal membranes, while the majority of the estrogen receptor remains soluble at all centrifugation speeds. Although these cells differ markedly in their estrogen receptor content and sensitivity to growth inhibition by the antiestrogen, tamoxifen, all three cell lines contain similar quantities of estrogen-noncompetible antiestrogen binding sites (MCF-7 cells, 390 ± 50 (S.E.); T47D cells, 360 ± 50; and MDA-MB-231 cells, 260 ± 50 fmol/mg protein) that have a similar affinity (Kd = 2 to 4 nM) for tamoxifen. The affinity of a series of antiestrogens and related compounds for these antiestrogen sites follows the order cis-tamoxifen > α-|p-[2-(1-pyrolidinyl)ethoxy]phenyl| 4-methoxy-α'-nitrostilbene (CI628) > trans-tamoxifen = trans-hydroxytamoxifen > α-|p-[2-(1-pyrolidinyl)ethoxy]phenyl| 4-hydroxy-α'-nitrostilbene (CI628M) > 6-hydroxy-2-(p-hydroxyphenyl)benzo(b)thien-3-yl-p- [2(1-pyrolidinyl)ethoxy]phenyl ketone (LY117018). This order of affinities of different antiestrogens for the antiestrogen binding sites does not parallel their affinity for the estrogen receptor nor the potency of these compounds as antiestrogens.

Recent studies have suggested that these estrogen-noncompetible sites in mediating directly the estrogen antagonism of antiestrogens in breast cancer cells, and suggest that interaction with the estrogen receptor is most likely the mechanism underlying the growth-inhibitory effects of antiestrogens. It is possible, however, that these antiestrogen binding sites might influence the distribution of antiestrogens and, hence, their accessibility to estrogen receptor in estrogen receptor-positive cells, or they might mediate actions of antiestrogens that are unrelated to estrogen antagonism.

INTRODUCTION

Antiestrogens are intriguing compounds that are able to antagonize many of the effects of estrogens. Although these nonsteroidal triphenylethylene compounds were developed initially by pharmaceutical companies as fertility-regulating agents, they are of particular interest and importance today because of their clinical efficacy in controlling the growth and spread of hormone-dependent mammary and uterine tumors; with them, it appears to be possible to achieve noninvasively the same hormonal effects and tumor regression that normally follow the more devastating endocrine ablative surgeries (ovariectomy, adrenalectomy, and hypophysectomy). Over the past 10 years, clinical trials using antiestrogens have documented that antiestrogen treatment is an effective endocrine therapy for breast cancer with fewer side effects than are associated with pharmacological hormone therapy (5, 13, 17, 18, 27, 41).

While the mechanism by which antiestrogens evoke their antitumor effects is incompletely understood, most experimental data are consistent with the hypothesis that antiestrogens exert their effects through the estrogen receptor system of target cells (8–10, 18, 22, 24, 27). Nonsteroidal antiestrogens bind directly to estrogen receptors in cytosol preparations from breast cancer cells, and these antiestrogen receptor complexes become localized in the nucleus (9, 10, 18, 19). Furthermore, the affinity of different antiestrogens for the estrogen receptor correlates well with their potency in inhibiting tumor cell growth (8, 10, 34). Hence, the estrogen antagonism of antiestrogens appears to be mediated, at least in large part, by antiestrogen interaction with the estrogen receptor system.

Recently, it has been reported that triphenylethylene compounds also bind to additional saturable sites present in the cytosol of rat and guinea pig uterus, chick oviduct, and human breast cancers (12, 15, 28, 36–39). These sites are distinct from the estrogen receptor and are most readily distinguishable by the fact that they bind triphenylethylene antiestrogens but, in contrast to the estrogen receptor, they do not bind steroidal or nonsteroidal estrogens (38). These sites have been termed “antiestrogen-specific” or “estrogen-noncompetible” binding sites. Since these sites are present in estrogen target tissues which are responsive to antiestrogen, they could be important in mediating and/or modulating the actions of antiestrogens.

To aid in defining the role of these antiestrogen binding sites in influencing the action of antiestrogens in breast cancer cells,
we have studied the distribution, quantities, and binding characteristics of these sites in 3 human breast cancer cell lines that differ in their estrogen receptor content and in their sensitivity to growth suppression by antiestrogens: MCF-7 cells contain high levels of estrogen receptor, and their growth is inhibited markedly by antiestrogens; T47D cells contain low levels of estrogen receptor, and their growth is inhibited weakly by antiestrogens; and MDA-MB-231 cells contain no detectable estrogen receptors, and their growth is unaffected by antiestrogens. Our results show that, despite marked differences in the responsiveness of these cells to growth inhibition by the antiestrogen, tamoxifen (1-[4-(2-dimethylaminoethoxy)phenyl]-1,2-diphenylbut-1-[Z]-ene), all 3 cell lines possess similar quantities of estrogen-noncomparable, antiestrogen binding sites with similar affinities for tamoxifen. In addition, the subcellular distribution of these antiestrogen binding sites and the relative binding affinities of various antiestrogens for these sites are similar in the 3 cell lines. These findings raise questions about the role of these sites in mediating directly the estrogen antagonistic effects of antiestrogens in breast cancer cells.

MATERIALS AND METHODS

Chemicals. The pure trans isomer of [3H]tamoxifen (12.2 Ci/mmol) used to assay the antiestrogen binding sites was synthesized by Dr. D. Robertson and Dr. J. Katzenellenbogen (32), University of Illinois, Urbana, Ill. Estrogen receptor content was assayed with [3H]estradiol (54 Ci/mmol) from New England Nuclear (Boston, Mass.). The nonradioactive antiestrogens, tamoxifen, and trans-4-hydroxytamoxifen (1-[4-(2-dimethylaminoethoxy)phenyl]-1,2-diphenylbut-1[Z]-ene), and the estrogen, cis-tamoxifen (ICI 47,699), were from ICI America, Inc. (Wilmingtorn, Del); CI628 was from Parke-Davis & Co. (Ann Arbor, Mich.); U23, 469, cis-[3-p-(1,2,3,4-tetrahydro-6-methoxy-2- phenyl)-1-naphthyl]phenoxyl]-1,2-propanedioil was from Upjohn Co. (Kalamazoo, Mich.); and LY117018 was obtained from Eli Lilly & Co. (Indianapolis, Ind.). The demethylated derivative of CI628, designated CI628M, was synthesized by Dr. D. Robertson and Dr. J. Katzenellenbogen (23). All media, sera, and antibiotics used to culture the cells were obtained from Grand Island Biological Co. (Grand Island, N. Y.). Insulin, hydrocortisone, estradiol, progesterone, testosterone, dihydrotestosterone, and diethylstilbestrol were purchased from Sigma Chemical Co. (St. Louis, Mo.).

Cells and Culture Conditions. MCF-7 cells were obtained from Dr. Charles McGrath of the Michigan Cancer Foundation (Detroit, Mich.) and were grown in plastic T-150 flasks in Eagle's minimal essential medium supplemented with 10% fetal bovine serum, gentamicin (50 μg/ml), penicillin (100 units/ml), streptomycin (100 μg/ml), insulin (1.5 ng/ml), and hydrocortisone (3.75 ng/ml), and 5% calf serum that had been treated with dextran-coated charcoal for 45 min at 55°C to remove endogenous hormones (CDCS) (10).

The T47D and MDA-MB-231 cells were obtained from EG & G Mason Research Institute (Worcester, Mass.). Medium for the T47D cells was the same as that for the MCF-7 cells except that the serum was 5% charcoal-treated fetal calf serum. The MDA-MB-231 cells were grown in Leibovitz's Medium L-15 supplemented with all of the additives used in the MCF-7 cell medium plus glutathione (16 mg/liter) and 5% calf serum that was not charcoal treated. Cell Growth Experiments. To determine the effect of tamoxifen on breast cancer cell growth, MCF-7 cells were seeded into T-25 flasks (1.5 x 10⁶ cells/flask) and grown for 2 days in medium supplemented as described above. After this time, cells from 2 flasks were harvested and counted with a Coulter Counter as described by Butler et al. (7) (Day 0). The medium was then changed to Eagle's minimal essential medium supplemented as described above except containing 2% CDCS and tamoxifen (10⁻⁸ M) or ethanol vehicle (0.1%). At several time points during the 12-day growth period, triplicate flasks of cells were counted. The procedure was similar for the T47D and MDA-MB-231 cells. T47D cells (seeded at 3 x 10⁵ cells/flask) were grown in regular culture medium throughout the growth period. MDA-MB-231 cells (seeded at 0.5 x 10⁵/3095 flasks) were grown in Leibovitz's Medium L-15 supplemented as described above but containing 2% CDCS.

Binding Studies. The cells from nearly confluent T-150 flasks were harvested by incubating cells at 37°C for about 10 min in Hanks' balanced salt solution (calcium and magnesium free; Grand Island Biological Co.) with 1 mM EDTA. The cells were washed twice with a buffer of either 5 mM sodium phosphate-10 mM thioglycollate-10% glycerol, pH 7.4, or TEA and homogenized in a Dounce homogenizer (40 to 50 strokes with a B-pestle).

In preliminary studies, the subcellular localization of the antiestrogen binding sites was determined by centrifuging MCF-7 cell homogenates at either 800 x g for 10 min; 12,000 x g for 30 min; or 100,000 x g for 60 min. In subsequent fractionation experiments, cell homogenates were sequentially centrifuged at 800, 12,000, and 180,000 x g. The 12,000 and 180,000 x g pellets were resuspended in buffer and thoroughly homogenized with a Kontes glass-glass homogenizer. For the Scatchard analysis and competition studies, the 12,000 x g supernatant was used.

The concentration of antiestrogen binding sites was determined by incubating 250 μl of cell supernatant or resuspended pellet with 5 μl of 150 nM [3H]tamoxifen in dimethylformamide and with 5 μl of ethanol or 10⁻³ M estradiol in 5 μl of ethanol. After a 30-min preincubation at 0°C to fill estrogen receptor sites, samples were diluted with 220 μl of TEA buffer prior to the addition of 20 μl of competitor in dimethylformamide as described previously (36). Samples were incubated for 16 hr at 0-4°C, and then 88 μl of dextran-coated charcoal (5% Norit A and 0.5% dextran in TEA buffer) was added. The charcoal was pelleted by centrifuging at 12,800 x g for 10 min, and an aliquot of the supernatant was removed for determination of bound radioactivity.

Estrogen receptor content was assayed in similar incubations containing 10⁻⁹ M radiolabeled estradiol in the presence and absence of 10⁻⁸ M unlabeled estradiol for 16 hr at 0-4°C and treating with dextran-charcoal as described above.

Enzyme Assays and Protein and DNA Determinations. The microsomal marker enzyme, glucose-6-phosphatase, was assayed by the method of Hubscher and West (20). Acid phosphatase which served as a lysosomal marker enzyme was assayed as described by Szego et al. (40). The P₄ liberated in these assays was analyzed using a kit for the colorimetric determination of inorganic phosphorus (Sigma). Glucose-6-phosphate dehydrogenase, a soluble cytoplasmic marker, was assayed by the method of Glock and McLean (14). Protein was determined by the method of Peterson (31). DNA was assayed by the diphenylamine reaction (6).

RESULTS

Subcellular Localization of Antiestrogen Binding Sites. Although other investigators have reported that estrogen-noncomparable binding of the antiestrogen, tamoxifen, occurs in cytosol preparations from MCF-7 cells (28, 39), we had always found antiestrogen and estrogen binding in cytosol to be nearly mutually competitive (9); we, therefore, found little evidence for antiestrogen-specific binding sites in our high-speed MCF-7 cytosol preparations. However, when we undertook subcellular fractionation studies, we found that substantial amounts of estrogen-noncomparable, antiestrogen binding sites do exist in low-speed supernatants.
When tamoxifen binding was investigated in homogenates of MCF-7 cells centrifuged at different speeds for various periods of time, "antiestrogen-specific" binding sites were present in the 800 and 12,000 × g for 30 min low-speed supernatants of cell homogenates, but were pelleted upon centrifugation at 100,000 or 180,000 × g for 60 min (Chart 1). Chart 1 shows the results of assays in which a trace amount of tritiated tamoxifen (1.5 nm) was incubated in the presence of increasing concentrations of unlabeled estradiol or unlabeled tamoxifen (after a 30-min pre-treatment with 10⁻⁶ M estradiol to occupy estrogen receptors sites fully). The difference between the estradiol curve and the tamoxifen plus 10⁻⁶ M estradiol curve is considered to represent the estrogen-noncompetitive, but antiestrogen-competitive (“antiestrogen-specific”) binding sites. The subcellular fractionation pattern for “antiestrogen-specific” sites differs from that of the estrogen receptor (Chart 1, right of each panel) which remained soluble at all centrifugation speeds. However, the 100,000 and 180,000 × g supernatants contain approximately 50 to 70% of the estrogen receptors in the 800 and 12,000 × g supernatant fractions and, therefore, some estrogen receptors may be pelleted at the higher centrifugation speeds (as noted by others for estrogen and androgen receptors) (25, 30).

It is important to note that, since the different subcellular fractions contain a different proportion of “antiestrogen-specific” binding sites and estrogen receptor sites, the distribution of the tritiated tamoxifen between these sites varies in the different supernatant preparations. Hence, the apparent increase in effectiveness of estradiol in competing for tritiated tamoxifen binding (estradiol curves) in the 100,000 and 180,000 × g supernatant preparations is most probably attributable to the relative increase in estrogen receptor content versus antiestrogen binding sites and nonspecific binding, and not to an increase in the affinity of tamoxifen for the receptor.

In an attempt to define the subcellular localization of antiestro-

![Graph](chart1.png)

Chart 1. Subcellular fractionation of antiestrogen binding sites and estrogen receptors from MCF-7 cells. Cells were homogenized, and the homogenate was centrifuged at the different speeds for the times indicated. After centrifugation, the supernatants (s/t) were incubated with [³H]tamoxifen (1.5 nm) alone (O), [³H]tamoxifen with increasing (10⁻⁸ to 10⁻⁶ M) concentrations of estradiol (E₂, O), or [³H]tamoxifen plus 10⁻⁶ M estradiol for 30 min at 0° to fill estrogen receptor sites (●) prior to addition of tamoxifen (Tam; 10⁻⁶ to 10⁻⁴ M; □). Incubations were maintained for 18 hr at 0° prior to charcoal-dextran treatment. Additional aliquots of the supernatants from each centrifugal fraction were incubated with [³H]estradiol (10 nm) in the absence and presence of 10⁻⁶ M unlabeled estradiol to monitor estrogen receptor levels (◼, ◼). Values are expressed as pmol [³H]-tamoxifen bound per mg cell DNA (left ordinate) and as pmol [³H]estradiol bound per mg cell DNA (right ordinate). Please note that the scale for antiestrogen binding sites is expanded for clarity in the 100,000 × g and 180,000 × g fractions.

![Graph](chart2.png)

Chart 2. Subcellular distribution of antiestrogen sites, estrogen receptors, and several enzyme markers upon sequential centrifugation of the 10-mm 800 × g MCF-7 supernatant. Levels of antiestrogen sites, estrogen receptors, and the activity of 3 enzyme markers were determined in the 10-mm 800 × g supernatant fraction. The 800 × g supernatant was then centrifuged at 12,000 × g for 30 min at 0°, and the level and activity of the antiestrogen sites, estrogen receptors, and enzyme markers were determined in the 12,000 × g supernatant and in the resuspended 12,000 × g pellet; 12,000 × g supernatant was then centrifuged at 180,000 × g for 1 hr, and these parameters were again monitored in the supernatant and resuspended pellet. In A, the total activity of each parameter in the 800 × g supernatant was set at 100% to allow a comparison with the subsequent distribution between supernatant and pellet fractions. In B, the relative specific activity (level or activity per mg protein) of each parameter in the 800 × g supernatant was set at 100% so that changes in specific activity during fractionation can be observed. The absolute specific activities of the 5 parameters in the 800 × g supernatant fraction were: antiestrogen sites, 290 fmol per mg protein; estrogen receptors, 336 fmol per mg protein; glucose-6-phosphatase, 13.0 μg P₃ released per mg protein per hr; acid phosphatase, 476 nmol α-naphthol per mg protein per hr; and glucose-6-phosphate dehydrogenase, 12.4 μmol NADPH per mg protein per min. Values are means of 2 separate fractionation studies. Bars, S.E.
pelleted. In contrast, most of the estrogen receptors and the soluble enzyme marker, glucose-6-phosphate dehydrogenase, remain in the 180,000 x g supernatant. The specific activity (sites/unit protein) of the antiestrogen sites is enriched in the 180,000 x g pellet (Chart 2B). Since the antiestrogen binding sites are pelleted by centrifugation at 180,000 x g for 60 min, they may be associated with the microsomal membranes. There is, however, little of the total activity (Chart 2A) of the microsomal marker enzyme, glucose-6-phosphatase, in the 180,000 x g for 60 min pellet. This enzyme and the lysosomal marker enzyme, acid phosphatase, are found predominantly in the 12,000 x g for 30 min pellet. The separation of the microsomal marker enzyme and antiestrogen binding sites, which also appear to be particulate, suggests that these 2 components may be located in different areas of the microsomal membranes which are known to be heterogeneous (1).

Antiestrogen Inhibition of Growth of Breast Cancer Cells and Antiestrogen Binding Sites in Different Breast Cancer Cell Lines. To determine if antiestrogen binding sites are present in other breast cancer cell lines, we examined tamoxifen binding in T47D and MDA-MB-231 cells as well as in MCF-7 cells. These 3 cell lines differ markedly in their estrogen receptor content and in their responsiveness to antiestrogen (Chart 3). Tamoxifen inhibition of growth was greatest in MCF-7 cells which we find to contain approximately 7 pmol of estrogen receptor/mg DNA. In T47D cells, estrogen receptor levels were 10 to 15% of the values determined for MCF-7 cells (data not shown), and tamoxifen decreased growth, but far less than in MCF-7 cells. No estrogen receptor was detected in MDA-MB-231 cells, and tamoxifen did not alter growth in these cells. Hence, tamoxifen inhibited growth only in those cell lines that contained estrogen receptor, i.e., MCF-7 and T47D cells, and the magnitude of growth suppression appears to be related to the estrogen receptor content of the cells.

In contrast to the differences observed in estrogen receptor levels and in responsiveness to tamoxifen among these 3 cell lines, the concentration of estrogen-noncompetible antiestrogen binding sites and their affinity for tamoxifen were found to be similar in the 3 cell lines (Table 1 and Chart 4). Chart 4 shows the results of a typical competitive binding experiment in which a trace amount of tritiated tamoxifen (1.5 nm) was incubated in the presence of increasing concentrations of unlabeled estradiol or unlabeled tamoxifen (after a 30-min pretreatment with 10^-6 M estradiol to fully occupy estrogen receptors). Values have been normalized per mg protein. These data are also replotted in the form of a Scatchard plot (Chart 4, lower panels) where Kd values of 2 to 4 nM are obtained for the binding of tamoxifen. As shown in Table 1, which summarizes data from 3 competitive binding experiments with each cell line, the antiestrogen-specific binding sites are present at approximately the same concentration in the 3 cell lines, and the affinity of tamoxifen for these sites is likewise similar in all of the cell lines.

Ligand Binding Specificity of Antiestrogen Binding Sites. Competitive binding assays were used to determine the affinities of various antiestrogens and related compounds for the "antiestrogen-specific" binding sites. As seen in Chart 5 and Table 2, these sites are specific for compounds which are triphenylethylene derivatives. None of the steroids (estradiol, hydrocortisone, progesterone, dihydrotestosterone, or testosterone) nor the nonsteroidal estrogen, diethylstilbestrol, competed with [3H]tamoxifen for binding to these sites. The order of competitive effectiveness is cis-tamoxifen > CI628 > trans-tamoxifen > trans-hydroxytamoxifen > CI628M > LY117018. Chart 5 also shows that the competitive binding curves are parallel for all of the triphenylethylene compounds tested. The competition curve for LY117018 has a somewhat shallower slope, suggesting that interaction of LY117018 with these antiestrogen sites may be
cells to growth suppression by tamoxifen are markedly different. In MCF-7 cells, the concentrations of estrogen receptor and "antiestrogen-specific" binding sites are approximately equal while, in T47D cells, the concentration of receptor is only one-eighth that of the antiestrogen sites. These 2 binding components are, however, readily distinguishable because: (a) the ligand binding specificities of the estrogen receptor and antiestrogen sites are very different; (b) the binding capacity of the estrogen receptor is destroyed by heating for 60 min at 37°C, while the antiestrogen sites are stable to this treatment (data not presented); and (c) the antiestrogen binding sites are pelleted by centrifugation at 180,000 × g for 60 min, whereas much of the estrogen receptor remains soluble.

The antiestrogen binding sites in human breast cancer cell lines appear to be similar to the antiestrogen binding sites described in rat tissues. We have recently reported that high-affinity (Kd 1 to 3 nm) estrogen-noncompetitive antiestrogen binding sites are present in the 12,000 × g min supematants of estrogen receptor-positive (uterus, ovary, liver, and brain) as well as estrogen receptor-negative (esophagus, lung, and spleen) rat tissues (36). In the immature rat uterus, the antiestrogen binding site content is about one-tenth that of the estrogen receptor. As in MCF-7 cells, these 2 binding components can be readily distinguished by differences in ligand specificity, fractionation pattern, and protease and heat sensitivity (36).

The antiestrogen binding sites we find in the 3 breast cancer cell lines are, in most respects (affinity, quantity, and ligand specificity), very similar to the estrogen-noncompetitive antiestrogen sites described by other investigators (11, 12, 15, 28, 37–39). However, our results differ in one respect from these other reports. Whereas others (11, 37–39) report that antiestrogen binding sites are present in the cytosolic fraction, we find that estrogen-noncompetitive binding sites are always pelleted by centrifugation at 180,000 × g for 60 min. Moreover, our fractionation experiments indicate that these sites may be membrane associated. If this is the case, differences in buffer toxicity and/or homogenization techniques may result in liberation of these sites to the supematant and, hence, account for the differences in apparent localization of these sites.

As reported by Sutherland et al. (38), the antiestrogen binding sites are specific for compounds with a triphenylethylene structure.

### Table 2

Affinities of antiestrogens and other compounds for estrogen receptor and antiestrogen binding sites of MCF-7 breast cancer cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>Estrogen receptor (%)</th>
<th>Antiestrogen binding sites (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estradiol = 100%</td>
<td>Trans-tamoxifen = 100%</td>
</tr>
<tr>
<td>Trans-tamoxifen</td>
<td>2 ± 0.5</td>
<td>100</td>
</tr>
<tr>
<td>cis-Tamoxifen</td>
<td>0.2 ± 0.1</td>
<td>267 ± 20</td>
</tr>
<tr>
<td>CI628</td>
<td>3 ± 2</td>
<td>156 ± 7</td>
</tr>
<tr>
<td>Trans-hydroxytamoxifen</td>
<td>167 ± 20</td>
<td>110 ± 8</td>
</tr>
<tr>
<td>CI628M</td>
<td>22 ± 4</td>
<td>60 ± 4</td>
</tr>
<tr>
<td>LY117018</td>
<td>115 ± 26</td>
<td>19 ± 8</td>
</tr>
<tr>
<td>Estradiol or dihydrotestosterone</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Progesterone or hydrocortisone</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Testosterone or dihydrotestosterone</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*All values determined by competitive binding assays using [3H]estradiol or [3H]tamoxifen for measurement of estrogen receptor or antiestrogen sites, respectively.*

*Mean ± S.E. of 3 determinations.*

*The competition curve with LY117018 has a shallower slope than that of trans-tamoxifen. This value has been determined from the concentration giving 50% competition.*

**DISCUSSION**

The results of our studies demonstrate that antiestrogen binding sites distinct from the estrogen receptor are present in the MCF-7, T47D, and MDA-MB-231 breast cancer cell lines. The concentration of the antiestrogen binding sites and their affinity for tamoxifen are similar in the 3 breast cancer cell lines, although the concentration of estrogen receptor and the sensitivity of the...
tecture. The affinity of different antiestrogens for these sites does not, however, correlate with their potency as antiestrogens. For example, cis-tamoxifen binds to these sites with twice the affinity of trans-tamoxifen but displays no estrogen-antagonistic properties (16, 21, 33). Other compounds (i.e., LY117018 and C1628M) show high antiestrogen potency and high estrogen receptor affinity (4, 9, 23, 34) but low or reduced affinity for the antiestrogen sites. Because these sites also have affinity for compounds that are estrogens (e.g., cis-tamoxifen), it is not fully appropriate to consider these as "antiestrogen-specific" binding sites.

Although the antiestrogen binding sites are present in all 3 cell lines, only those cells which contained estrogen receptors showed growth inhibition following tamoxifen treatment. This result, coupled with the finding that the affinity of antiestrogens for these sites does not parallel antiestrogenic potency, suggests that antiestrogen binding to these sites probably does not mediate the effects of antiestrogens on cell growth.

While our data shed doubt on the role of these antiestrogen sites in the growth suppression evoked by antiestrogens, it is conceivable that a defect in a biochemical process that occurs after tamoxifen binding to the antiestrogen sites could account for the tamoxifen resistance of cells, such as the MDA-MB-231 cells, that contain antiestrogen binding sites but do not display growth inhibition by tamoxifen. Surely this is possible, since there is clear evidence for the presence of estrogen receptors in some human breast cancers that are unresponsive to estrogens and antiestrogens (26, 29). In addition, Faye et al. (11) have presented evidence for a variant of MCF-7 cells that appears to have lost antiestrogenic properties (16, 21, 33). Other compounds (i.e., LY117018 and C1628M) show growth inhibition following tamoxifen treatment. This evidence for a variant of MCF-7 cells that appears to have lost antiestrogen binding sites in rat uterine cytosol. Biochem. Biophys. Res. Commun., 93: 1225–1231, 1980.


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