ABSTRACT

In eight estrogen receptor (ER)-positive breast cancer cell lines (including three sublines of MCF-7) and five ER-negative breast lines, the action of the nonsteroidal antiestrogen, tamoxifen, was studied, and the concentrations of ER and antiestrogen binding site were measured. The concentration of antiestrogen binding site was significantly \( P < 0.005 \) greater in ER-positive cells \([236,600 \pm 29,900 \text{(SE)} \text{ sites/cell}] \) than in ER-negative cell lines \([66,600 \pm 16,800 \text{ sites/cell}] \).

In ER-positive cell lines, a cell cycle phase-specific growth-inhibitory effect, 20% inhibitory dose \(< 0.1 \text{ to } 1.0 \mu \text{M} \), was seen which was shown for some representative cell lines to be estrogen reversible. Within this group of cell lines, the degree of tamoxifen-induced inhibition of growth correlated with control population doubling time, but not ER or antiestrogen binding site concentration. The changes in cell cycle kinetic parameters characteristic of all ER-positive lines were a decrease in percentage of S-phase cells and a corresponding increase in percentage of \( S \)-phase cells.

All in cell lines, 5 to 12.5 \( \mu \text{M} \) tamoxifen caused cytotoxicity, and this was shown to be estrogen-reversible in 3 representative cell lines; moreover, estradiol synergistically enhanced the cytotoxic effects of tamoxifen under some experimental conditions. The cell cycle effects of tamoxifen in three ER-negative cell lines \((\text{Hs}0578 \text{T}, \text{MDA-MB-231}, \text{MDA-MB-330}) \) were decreased proportions of \( G_0 \)-\( G_1 \) cells with an increase in percentages of \( S \) and \( G_2 \)-\( M \)-phase cells. These results implied that the mechanism of tamoxifen cytotoxicity may differ in ER-positive and ER-negative breast cancer cells. However, although the ER-negative BT-20 line was much less sensitive to tamoxifen than were the ER-positive cells, growth inhibition and cytotoxicity in this line were associated with a slight decrease in percentage of S-phase cells.

These results confirm that ER-positive breast cancer cells are more sensitive (4- to >75-fold) than ER-negative breast cells to the growth-inhibitory effects of tamoxifen and demonstrate that, in all ER-positive cells, growth inhibition and cytotoxicity are accompanied by characteristic changes in cell cycle kinetic parameters. In contrast, different mechanisms may be involved in the effects of tamoxifen on different ER-negative cell lines.

INTRODUCTION

Recent studies from this laboratory on the action of tamoxifen in the ER\(^*\)-containing human breast cancer cell lines, T-47D and MCF-7, have shown that tamoxifen and its major metabolites exert both an estrogen-reversible cell cycle phase-specific inhibition of proliferation and, at higher doses, an estrogen-irreversible cytotoxic effect \((29, 30, 34, 35) \). Exponentially growing control MCF-7 cultures were shown by cell kinetic experiments to contain 2 populations of cells distinguishable by their rates of efflux from \( G_0 \)-\( G_1 \) phase; the majority of cells were "rapidly cycling" and left \( G_0 \)-\( G_1 \) with a mean \( t_M \) of 2.3 h, and a minority were "slowly cycling" with a mean \( G_0 \)-\( G_1 \) exit \( t_M \) of 28 h \((35) \). Following treatment with tamoxifen, there was an increase in the proportion of cells in the \( G_0 \)-\( G_1 \) phase of the cell cycle, with a corresponding decrease in the proportions of \( S \) and \( G_2 \)-\( M \) cells, due principally to an increase in the proportion of "slowly cycling" cells \((35) \). Experiments in which MCF-7 cells synchronized by mitotic selection were treated with tamoxifen for short intervals showed that the effects of tamoxifen were exerted primarily during a 2- to 4-h period in mid-\( G_1 \) phase \((39) \).

In the experiments reported in this paper, we have assessed the effects of tamoxifen on cell proliferation and cell cycle kinetics of a number of ER+ and ER- breast cancer cell lines in order to assess the generality of the observations made with MCF-7 and T-47D cells. Since there have been reports of heterogeneity among MCF-7 cell lines \((12, 32) \), the cells tested included 3 MCF-7 sublines. In addition, the relationship of the cellular concentration of high-affinity AEBS \((23, 36, 40) \) to response to tamoxifen was also investigated.

MATERIALS AND METHODS

Cell Lines. The 6 ER+ breast cancer cell lines used were ZR-75-1 (6), MCF-7 (33), MDA-MB-134 (5), T-47D (14), BT-474 (15), and MDA-MB-361 (4). The ER- lines consisted of 4 breast cancer lines, BT-20 (16), Hs0578T (10), MDA-MB-330 (4), and MDA-MB-231 (5) and one line derived from lactating breast, HBL-100 (7). MCF-7 sublines were obtained from 3 sources: E. G. and G. Mason Research Institute, Worces-
ter, MA, for the National Cancer Institute Breast Cancer Program Cell Bank (designated MCF-7-M); Dr. M. E. Lippman, Medicine Branch, National Cancer Institute, N.I.H., Bethesda, MD (MCF-7-L); and Dr. C. McGrath, Meyer L. Prentis Cancer Center, Detroit, MI (MCF-7). All other cell lines were obtained from E. G. and G. Mason Research Institute at the passage numbers listed in Table 1.

In view of evidence that the magnitude of the effect of tamoxifen on both MCF-7 \((3, 35) \) and T-47D \((30) \) cells depended on the cell culture conditions, as far as possible, the same cell culture conditions were used for all cell lines tested. Aliquots of 10\(^6\) cells in 2 ml of medium (see below) containing 200 \( \mu \text{M} \) of dimethyl sulfoxide were stored in liquid nitrogen. Cells were revived by thawing, and stock cultures were maintained in continuously exponential growth by weekly passage of the appropriate numbers of cells following trypsinization of the monolayers with 0.05% trypsin:0.02% EDTA in phosphate-buffered saline (1.5 \( \text{mM} \) \( \text{KH}_2\text{PO}_4:8.1 \text{mM} \text{Na}_2\text{HPO}_4:2.7 \text{mM} \text{KCl}:140 \text{mM} \text{NaCl}) \). Stock cultures were grown in RPMI Medium 1640 supplemented with 5 \( \text{mM} \) glutamine, 14 \( \text{mM} \) sodium bicarbonate, 20 \( \text{mM} \) 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer, insulin \((10 \text{ \mug/ml})\), gentamicin \((20 \text{ \mug/ml})\), and 10% FCS.

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Mycoplasma contamination was monitored by routine assay tech-
niques, and only Mycoplasma-free cultures were used.

Drug Treatment. Tamoxifen [trans-1-(4-|3-dimethylaminoethoxy-
phenyl)-1,2-diphenylbut-1-ene] and 17β-estradiol were obtained and
stock solutions prepared in ethanol as described previously (34).

Experiments were carried out using cells grown in medium which
differed from that used for maintaining stock cultures only in that it
was supplemented with 5% rather than 10% FCS. Cultures were inoculated at a density of 5 x 10^5 cells/25-cm² flask containing 5 ml of medium.
When cell numbers reached 6.10^5, the medium was replaced with
medium containing 0.1 to 12.5 µM tamoxifen or 0.1% ethanol vehicle.

For a few cell lines (MCF-7, MDA-MB-231, and BT-20), flasks were
harvested at 3 or 4 time points, and for all cell lines, flasks were harvested
after approximately 4 control population doublings. Viable cells were
counted using a hemocytometer and phase-contrast microscopy.

Mean doubling times were calculated from the initial and final cell
numbers for each tamoxifen concentration, and the results were
expressed as:

\[
\text{Doubling time (control)} = \frac{\text{Doubling time}}{(\text{doubling rate})}
\]

in order to account for the variation in number of control population
doublings among the cell lines tested.

In selected cell lines, the ability of 17β-estradiol to reverse the effects
of tamoxifen on cell numbers was assessed by the simultaneous addition
of tamoxifen and 17β-estradiol to the
medium, and in the case of 7.5 µM tamoxifen, 17β-estradiol was added
to >75-fold between the majority of ER+ and ER- lines. However,
to 1.0 µM tamoxifen. In contrast, the ER- cell lines had ID_{20} concentrations of <0.1
µM tamoxifen. In contrast, the ER- cell lines had ID_{20} concentrations of <0.1

RESULTS

The assignment of breast cancer cells as ER+ or ER- in this
study (Table 1) is in agreement with other reports except for the
BT-474 line, which had previously been found by Lasfargues et
al. (15) to be ER- in a study described by the authors as
preliminary. ER+ breast cancer cell lines had significantly (P <
0.005) greater AEBs concentrations (236,600 ± 29,900 sites/cell;
mean ± SE) than the ER- lines (66,600 ± 16,800 sites/cell)
(Table 1). There appeared, therefore, to be 2 groups of cell lines
according to their ER status, AEBs concentration, and sensitivity
to tamoxifen (Table 1; Chart 1).

ER+ breast cancer cell lines had ID_{20} concentrations of <0.1
10 µM tamoxifen. In contrast, the ER- cell lines had ID_{20} of 4
to >7.5 µM, i.e., there was a difference in sensitivity of 4- to
>75-fold between the majority of ER+ and ER- lines. However,
the MCF-7 subline, MCF-7-L (Chart 2) had intermediate sensitivity
in tamoxifen (ID_{20} = 2.0 µM) under these experimental
conditions.

For 3 representative cell lines, i.e., an ER+ line (MCF-7) and 2
ER- lines (MBA-MB-231 and BT-20), the time course of tamoxif-
en action on cell numbers was studied (Chart 3). Since these
growth curves (except for 10 µM tamoxifen in Chart 3A) were
approximately linear, they are adequately described by a single
parameter such as the mean proliferation rate. For 10 µM tamoxi-
fen, the growth curves were more complex, however, with some
increase in cell numbers occurring in the first 24 h, followed by
a rapid decline in numbers of ER+, MCF-7 cells (Chart 3B) and the decrease seen after

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Table 1

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Passage at supply</th>
<th>Concentration (10^3 x sites/cell)</th>
<th>K_d (nm)</th>
<th>Concentration (10^4 x sites/cell)</th>
<th>K_d (nm)</th>
<th>Published ER status</th>
<th>ID_{20}a (µM)</th>
<th>&quot;Cytotoxic&quot; concentrationa (µM)</th>
<th>Doubling timeb (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZR-75-1</td>
<td>299 (329)</td>
<td>2.79 ± 0.65</td>
<td>0.09 ± 0.02</td>
<td>3.29 ± 0.51</td>
<td>1.3 ± 0.06</td>
<td>(+)</td>
<td>11.0</td>
<td>10</td>
<td>28</td>
</tr>
<tr>
<td>MCF-7</td>
<td>88.5</td>
<td>9.51 ± 0.15</td>
<td>0.10 ± 0.02</td>
<td>1.41 ± 0.20</td>
<td>0.97 ± 0.15</td>
<td>(+)</td>
<td>11.0</td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td>MCF-7-L</td>
<td>145 (152)</td>
<td>1.53 ± 0.25</td>
<td>0.08 ± 0.01</td>
<td>2.57 ± 0.41</td>
<td>1.14 ± 0.01</td>
<td>(+)</td>
<td>25,26</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td>MDA-MB-134</td>
<td>42 (52)</td>
<td>2.47</td>
<td>0.17</td>
<td>3.12 ± 0.17</td>
<td>1.26 ± 0.15</td>
<td>(+)</td>
<td>12,14</td>
<td>10</td>
<td>22</td>
</tr>
<tr>
<td>T-47D</td>
<td>74 (92)</td>
<td>4.41 ± 0.66</td>
<td>0.11 ± 0.01</td>
<td>0.99 ± 0.21</td>
<td>1.07 ± 0.15</td>
<td>(+)</td>
<td>12,14</td>
<td>10</td>
<td>35</td>
</tr>
<tr>
<td>BT-24</td>
<td>28 (33)</td>
<td>1.89 ± 0.70</td>
<td>0.18 ± 0.05</td>
<td>2.14 ± 0.01</td>
<td>0.96</td>
<td>(+)</td>
<td>15</td>
<td>5</td>
<td>89</td>
</tr>
<tr>
<td>MDA-MB-361</td>
<td>23 (30)</td>
<td>2.07 ± 0.59</td>
<td>0.11 ± 0.02</td>
<td>2.23 ± 0.27</td>
<td>1.3 ± 0.15</td>
<td>(+)</td>
<td>12 &lt;0.01</td>
<td>7.5</td>
<td>75</td>
</tr>
<tr>
<td>BT-20</td>
<td>288 (296)</td>
<td>0.10 ± 0.08</td>
<td>0.75</td>
<td>(12)</td>
<td>5.0</td>
<td>7.5</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heo578</td>
<td>14 (23)</td>
<td>0.48 ± 0.15</td>
<td>0.88</td>
<td>(2)</td>
<td>7.1</td>
<td>10</td>
<td>76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA-MB-330</td>
<td>21 (29)</td>
<td>0.40 ± 0.22</td>
<td>0.95 ± 0.22</td>
<td>0.12 &gt;7.5</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBL-100</td>
<td>18 (27)</td>
<td>0.27</td>
<td>0.71</td>
<td>(12)</td>
<td>7.8</td>
<td>12.5</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>0</td>
<td>0.82</td>
<td>0.65</td>
<td>(2,12)</td>
<td>7.8</td>
<td>12.5</td>
<td>33</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Measured as described in "Materials and Methods."
b Tamoxifen concentration at which a 20% reduction in cell population doubling rate occurred, determined from the data presented in Charts 1 and 2.
c Lowest tamoxifen concentration at which an absolute reduction in cell numbers occurred.
d Mean of at least 3 determinations under the conditions described for Charts 1 and 2.
e Mean ± SE or range.
f Numbers in parentheses, reference.
g Numbers in parentheses, passage at experiment.

ER Assay. Total salt-extractable ER content was measured as de-
scribed previously (29). Briefly, cells were grown to subconfluence,
harvested, washed, homogenized in 10 mM Trism:1.5 mM EDTA buffer,
pH 7.4 containing 1 mM NaCl, and a charcoal:dextran-treated high-speed
supernatant was obtained. ER concentration was determined by satu-
ratron analysis at 4°C for 16 to 20 h using increasing concentrations of
17β-[3H]estradiol (0.1 to 5 nM). Bound and unbound ligand were sepa-
rated by dextran-charcoal adsorption, and after correction for nonspecific
binding, the data were analyzed by the method of Scatchard (31). Such
a technique measures unoccupied ER only.

AEBS Assay. The high-affinity microsomal binding site for tamoxifen
was assayed as described previously (40).

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SENSITIVITY OF-breast cell lines to tamoxifen

Within the group of ER+ breast cancer cell lines and sublines, sensitivity to tamoxifen did not correlate with either unoccupied ER or AEBS concentrations (Table 1), but there was a linear correlation between percentage decrease in proliferation rate at a given tamoxifen concentration (e.g., 0.1, 0.5, or 1 μM) and control population doubling time (coefficients of determination; ρ2 = 0.61, 0.60, and 0.60, respectively). Within the group of ER- cell lines also, AEBS concentration did not correlate with sensitivity to tamoxifen.

In contrast to the wide range of ID50 concentrations (<0.1 to >7.5 μM tamoxifen), cytotoxicity occurred over a much smaller tamoxifen concentration range (5 to 12.5 μM), but tended to occur at lower concentrations in ER+ lines (5 to 10 μM) than in ER- lines (7.5 to 12.5 μM (Table 1)).

The cell cycle kinetic changes induced by tamoxifen differed between ER+ and ER- cell lines. In all of the ER+ cell lines (Chart 4A) and the MCF-7 sublines (data not shown), tamoxifen induced a dose-dependent decrease in the proportion of S-phase cells, accompanied, as illustrated in Chart 5A (and Refs. 9, 29, 30, 34, and 35), by a corresponding increase in the proportion of G0-G1 phase cells. In contrast, 3 of the ER- lines (Hs0578T, MDA-MB-231, and MDA-MB-330) showed an increase in percentage of S-phase cells (Chart 4B) accompanied by a decrease in percentage of G0-G1 cells, and an increase in percentage of G2-M cells (Chart 5C). In one of the ER- lines (BT-20), there was a slight but significant (P < 0.01) fall in percentage of S-phase cells, and in another (HBL-100), the DNA histograms were virtually unchanged over the tamoxifen concentration range tested (Charts 4B and 5B).

A 10-fold lower concentration of 17β-estradiol was able to reverse the tamoxifen-induced cytostasis either fully (1 μM tamoxifen) or partially (2.5 to 7.5 μM tamoxifen) in the ER+ line tested (T-47D; Chart 6A), but this did not occur in the 2 ER- cell lines (MDA-MB-330 and HBL-100; Chart 6, B and C). Tamoxifen-
SENSITIVITY OF BREAST CELL LINES TO TAMOXIFEN

induced cytotoxicity was not reversed by 17β-estradiol in any cell line tested (Chart 6). Estradiol concentrations up to 1 μM had no effect on proliferation rate in any line (Chart 6).

For 7.5 μM tamoxifen, the effect of simultaneous addition of 17β-estradiol over a 1000-fold concentration range (7.5 nM, 75 nM, 750 nM or 7.5 μM) was tested. Tamoxifen-induced growth inhibition was partially reversed by 7.5 to 750 nM 17β-estradiol in ER+, T-47D cells (Chart 7A), but not in the MDA-MB-330 or HBL-100 lines (Chart 7, B and C). In contrast, 7.5 μM 17β-estradiol was synergistic with 7.5 μM tamoxifen in inhibiting the growth of all 3 cell lines, although 7.5 μM 17β-estradiol alone produced no (T-47D) or minimal (3.4%, not significant, MDA-MB-330; 6.3%, P < 0.01, HBL-100) decrease in proliferation rate (Chart 7).

DISCUSSION

This study is in general agreement with other studies which have shown differences in the response to tamoxifen of ER+ and ER− breast cancer cells in vitro (8, 18, 20) and confirms earlier reports from this laboratory (29, 30, 34, 35, 39) that tamoxifen may exert both an estrogen-reversible cell cycle phase-specific growth-inhibitory effect and estrogen-irreversible cytotoxicity at higher concentrations. In this study, a complete correlation has been shown between the occurrence of the estrogen-reversible cell cycle phase-specific growth-inhibitory effect of tamoxifen and the presence of ER in human breast cancer cells. These data are consistent with the hypothesis that this effect of tamoxifen is mediated through an ER-related mechanism and appears to reflect the clinically observed correlation between ER status and response of breast tumors to tamoxifen (1, 19, 22). The clinical correlation between ER status and response to endocrine therapy, however, is not complete, and reasons advanced in explanation include limitations of assay procedures (28), variation in ER content even between histologically comparable portions of the same tumor (11), problems of collection and storage of tumor specimens (24), and measurement of cytoplasmic ER only (17).

The clear-cut distinction seen in this study between tamoxifen response in ER+ and ER− breast cancer cell lines appears to
SENSITIVITY OF BREAST CELL LINES TO TAMOXIFEN

Chart 5. Effects of tamoxifen on DNA histograms of selected human breast cancer cell lines. Cells were grown as described for Chart 1, and DNA flow cytometry and cell cycle analyses carried out as described in "Materials and Methods." Each DNA histogram is labeled with the tamoxifen concentration. The peak at Channel 10 to 20 represents chicken RBC added as an internal biological standard. C.V., coefficient of variation of the G0-G1 peak.

Chart 6. The effect of simultaneous administration of a 10-fold lower concentration of estradiol on the tamoxifen-induced inhibition of cell proliferation. Cells were treated as for Chart 1, but with or without a 10-fold lower dose of 17β-estradiol or 17β-estradiol alone. A, T-47D (ER+ breast carcinoma). B, MDA-MB-330 (ER+ breast carcinoma). C, HBL-100 (ER- cell line from normal lactating breast). Columns, mean of triplicate flasks expressed as a percentage of cell numbers in control cultures; bars, SE.

be at variance with a preliminary report from this laboratory (9) in which the dose relationship of the tamoxifen effect on cell numbers and cell cycle kinetic parameters was compared in the

ER+, MCF-7 and ER-, BT-20 breast cancer lines and shown to be similar. The design of the experiment, however, included a tamoxifen concentration range of 1 to 10 μM and culture medium containing 1% charcoal-treated FCS. Subsequent experiments have shown that the use of charcoal-treated FCS minimizes the responsiveness of ER+ cells to tamoxifen, and lowering the

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concentration of FCS to 1% shifts the dose-response curve and markedly increases drug sensitivity (30). The net result of such an experimental design (9) was to markedly reduce the differential sensitivity of these cell lines to tamoxifen and cause a convergence of their dose-response curves. Further, on the basis of the cell cycle kinetic data presented in Chart 4B, it appears that the BT-20 line has a qualitatively similar response to the ER+ cell lines which confirms our earlier observation (9). However, the present study would suggest that such changes are not typical of ER− breast cancer lines in general. Thus, unlike ER+ cells, different ER− lines may show different cell cycle changes. Since this implies different mechanisms of action in different ER− lines, further investigations in this area seem warranted.

Although there was complete correlation in the present study between the presence of ER and the occurrence of growth inhibition at low tamoxifen concentrations, there was no correlation between the amount of ER present and degree of sensitivity within the ER+ group (Table 1). The interpretation of a recent study (20) which correlated the ER concentration with the tamoxifen sensitivity of MCF-7 and T-47D cells is complicated by the fact that the 2 lines were grown under different cell culture conditions, i.e., different types and concentrations of calf serum and we have shown that this may affect response to tamoxifen (30). Clinical studies which have shown a relationship between quantity of tumor ER and likelihood of a response to tamoxifen (1, 19) are not directly relevant, since what is reported is presence or absence of response rather than some measure of the amount or rate of such response.

Within the ER+ group of cell lines, however, sensitivity to tamoxifen did correlate with control population-doubling time (Table 1). A previous study reported from this laboratory showed that the effects of tamoxifen on the proliferation of MCF-7 cells were principally confined to a short period in mid-G1 phase of the cell cycle (39). The most variable portion of the cell cycle is the G1 phase, so in general a prolonged cell cycle time implies a long G1, and perhaps that portion of G1 in which the cells are sensitive to tamoxifen is also increased relative to the total cell cycle time resulting in an enhanced tamoxifen effect. An alternative explanation may be that the more slowly growing cell populations may have a greater proportion of cells per cycle undergoing cell death, so that the number of cell generations per population doubling is greater, and the observed increase in sensitivity is only apparent. In any case, it is of interest that in several studies in which MCF-7 cell growth was inhibited by concentrations of tamoxifen lower than seen in the present study, the control population-doubling times were relatively prolonged (18, 25).

There is evidence that there are differences in the MCF-7 cell lines maintained in various laboratories (12, 27). It was perhaps not surprising, therefore, that the MCF-7 sublines showed some differences in tamoxifen sensitivity (Chart 2) and ER content (Table 1).

Evidence for antiestrogen-induced cytotoxicity in a number of experimental systems both in vitro and in vivo has recently been summarized (37). In the present study, the definition of the "cytotoxic concentration" as that at which an absolute decline in cell numbers occurred (Table 1) is somewhat arbitrary, since such an effect could theoretically be produced by the combination of a drug-induced total inhibition of cell proliferation together with an unaltered intrinsic cell death rate. In a majority of the cell lines tested, however, there was a tamoxifen concentration of \( \leq 12.5 \mu M \) at which the rate of decline in cell numbers exceeded the rate of increase of control populations (data not shown) thus providing unequivocal evidence for the occurrence of tamoxifen-induced cytotoxicity. Because of the steepness of this region of the dose-response curve (Chart 1), the "cytotoxic concentration" as defined provided a convenient and sufficiently accurate estimate. Although "cytotoxicity" occurred at slightly lower concentrations in ER+ cell lines, this difference may be only apparent since, in the ER+ lines, but not ER− lines, cytotoxicity was superimposed on the growth-inhibitory effect which would tend to cause "cytotoxicity" to become apparent at a lower concentration of tamoxifen. The observation that tamoxifen caused cytotoxicity in both ER+ and ER− lines confirms earlier reports (8, 9), and suggests that ER is not essential for the occurrence of tamoxifen-induced cytotoxicity.

We have discussed previously the possibility that cytotoxicity observed in vitro may have a correlate in vivo (29). The observation that cytotoxicity occurred in all cell lines in the present study suggests that, unless there is some mechanism whereby tamoxifen is effectively accumulated to cytotoxic concentrations in ER+ breast tumors but not in other tissues, tamoxifen-induced cytotoxicity may be unlikely to have any relevance to the situation in vivo. Thus, both the mechanism of antiestrogen-induced cytotoxicity in vitro, and its relevance to the regression of tumors in vivo, remain open questions.

The cell cycle kinetic effects of tamoxifen were different in ER+ and the majority of ER− breast cancer cells (Charts 4 and 5). The basis of the effects in ER+ cells, i.e., an accumulation of cells in G2−M phase, has been shown to be due to an increase in the proportion of "slowly cycling" cells (35). The effects seen in 3 of the ER− cell lines (Hs0578T, MDA-MB-231, and MDA-MB-330), i.e., an accumulation of cells in S-phase, with a corresponding decrease in G2−M cells could indicate either selective killing of cells in G2−M phase, or a prolongation of the S-phase transit time. In addition, the accumulation of cells in G2−M, in some cell lines, e.g., MDA-MB-231, may indicate a tamoxifen-induced defect in nuclear division.

Interestingly, the cell cycle kinetic effects of tamoxifen on these 3 ER− lines resemble to some extent those of pharmacological concentrations of 17β-estradiol. Evidence against the proposition that tamoxifen is merely exerting a high-dose estrogenic effect in ER− cells is the fact that 7.5 \( \mu M \) tamoxifen was more potent than equimolar 17β-estradiol and that 7.5 \( \mu M \) 17β-estradiol and tamoxifen were synergistic in decreasing cell numbers (Chart 7), suggesting that their modes of action are at least partly different. Although pharmacological doses of estrogens have been used in the treatment of breast cancer for many years, there are few data on their mechanism of action (37).

This study also demonstrated the presence of AEBS in all cell lines studied. Concentrations in MCF-7 cells were higher than previously reported, presumably because microsomal AEBS was measured [cf. cytosolic AEBS in earlier studies (13, 23)], and a different assay technique was used (20). Interestingly, concentrations were significantly greater in ER+ cells and while the physiological basis of this is not understood, it may relate to the

R. R. Reddel and R. L. Sutherland, unpublished observations.
recent observation that AEBS is an estrogen-induced protein in some tissues (41).

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Differential Sensitivity of Human Breast Cancer Cell Lines to the Growth-inhibitory Effects of Tamoxifen

Roger R. Reddel, Leigh C. Murphy, Rosemary E. Hall, et al.


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