Effects of Tamoxifen on Human Breast Cancer Cell Cycle Kinetics: Accumulation of Cells in Early G\textsubscript{1} Phase

C. Kent Osborne, David H. Boldt, Gary M. Clark, and Jeffrey M. Trent

Department of Medicine, University of Texas Health Science Center, San Antonio, Texas 78284 [C. K. O., D. H. B., G. M. C.], and the University of Arizona Health Science Center, Tucson, Arizona 85724 [J. M. T.]

ABSTRACT

We have studied the effects of tamoxifen on the cell cycle kinetics of the endocrine-responsive MCF-7 human breast cancer cells. Tamoxifen inhibits proliferation of MCF-7 cells. The tritiated thymidine labeling index is markedly reduced by tamoxifen, indicating a reduction in the fraction of cells in S phase. Flow cytometry of mithramycin-stained cells reveals that cells accumulate in G\textsubscript{1} phase, with a concomitant depletion of S- and G\textsubscript{2}-M-phase cells with tamoxifen. Mapping of G\textsubscript{1}-phase cells by morphology of prematurely condensed chromosomes demonstrated that tamoxifen-treated cells accumulate in early G\textsubscript{1}. These studies indicate that tamoxifen inhibits proliferation of MCF-7 human breast cancer cells by invoking a transition delay early in the G\textsubscript{1} phase of the cell cycle.

INTRODUCTION

The antiestrogen tamoxifen is now widely used for the treatment of patients with breast cancer. However, its mechanism of action is not totally defined. Although specific binding sites for tamoxifen have been identified recently in certain tissues (15), tamoxifen, like other antiestrogens, is thought to exert its effects by binding to estrogen receptor and then translocating it to the nucleus, where steps leading to estrogen-regulated events such as tumor growth are inhibited (6). Tamoxifen has been shown to inhibit breast tumor growth in experimental animals (8) and to inhibit a variety of biochemical events in cultured human breast cancer cells including nuclear processing of estrogen receptor (15), inhibition of a variety of biochemical events in cultured human breast cancer cells by invoking a transition delay early in the G\textsubscript{1} phase of the cell cycle.

MATERIALS AND METHODS

Cell Proliferation. The tissue culture methodology used for propagation of the MCF-7 human breast cancer cells has been described previously (14). For determination of the effects of tamoxifen on cell proliferation, MCF-7 cells were replated into multiwell culture dishes in tissue culture medium (Richter's Improved Eagle's Minimal Essential Medium ZO; Grand Island Biological Co., Grand Island, N. Y.) supplemented with insulin (1.0 nm) and 5% bovine serum. After 24 hr, plating medium was replaced with medium supplemented with 5% dextrancoated charcoal-stripped bovine serum to reduce endogenous estradiol levels. As an additional 24 hr, tamoxifen (1 \muM) or ethanol (final concentration, 0.1%) was added to the dishes. At the indicated times, cells were suspended and counted in a hemocytometer.

RESULTS

When cells from the well-characterized MCF-7 estrogen recep...
tor-positive human breast cancer cell line were incubated with tamoxifen (Chart 1), inhibition of cell proliferation similar to that observed in previous reports (9, 17) was observed. In 5% charcoal-stripped serum, control cultures proliferated rapidly and had reached confluence by Day 6. Tamoxifen (1 μM) slowed the rate of proliferation slightly during the first 48 hr. Thereafter, proliferation was markedly reduced, cell number nearly plateaued, and the monolayers remained at a subconfluent density. These data suggest that more than one passage through the cell cycle is required for expression of maximal effects of tamoxifen at these concentrations. When MCF-7 cells were incubated with tamoxifen for longer durations or were incubated with tamoxifen in serum-free medium, cells would begin to detach from the monolayer and appear as floaters. It is important to emphasize, however, that, under the experimental conditions described in Chart 1, cell number did not decline with tamoxifen. These data suggest that tamoxifen slowed proliferation but did not have an immediate cytotoxic effect on the MCF-7 cells.

We next examined the effect of tamoxifen on the distribution of MCF-7 cells in the cell cycle. The TLI was used to estimate the fraction of cells in the S phase of the cell cycle (Chart 2). The TLI of control cells growing in medium supplemented with dextran-coated charcoal-stripped calf serum was 35% at the beginning of the experiment. The TLI decreased slightly over 96 hr in control cultures, probably reflecting depletion of nutrients from the medium and/or density-dependent growth inhibition as the monolayer neared confluence. Tamoxifen resulted in a marked reduction in the TLI, and by 72 to 96 hr, less than 10% of the cell population was in S phase.

To determine the effects of tamoxifen on the distribution of MCF-7 cells in the other cell cycle phases, DNA histograms were obtained by flow cytometry of mithramycin-stained cells (Chart 3). After 72 hr in control medium, 72% of cells were in GІ as revealed by computer analysis of DNA histograms, 19% were in S phase (similar to the TLI result), and 9% were in G2-M phases. Tamoxifen treatment resulted in a marked accumulation of cells in GІ (92%), concomitant with a depletion of cells from the S and G2-M compartments. When experiments were carried out for longer time periods (6 days), cells exposed to tamoxifen remained in GІ, indicating that the block in GІ phase was not temporary.

It has been reported that serum-deprived or plateau-phase normal (nontransformed) cells accumulate in early GІ, whereas malignant or transformed cell populations accumulate in late GІ (4). Furthermore, certain metabolic inhibitors, such as actinomycin D or cycloheximide, block cells in early GІ in contrast to inhibitors of DNA synthesis, such as thymidine or hydroxyurea, which block cell cycle progression at the GІ-S boundary (4). Thus, we were interested in determining where in the GІ phase the tamoxifen block occurred. Utilizing the morphology of PCC, cells can be classified as occurring early or late in the GІ phase based upon the structural criteria of Hittleman and Rao (4). This is possible because the chromosomes from early GІ, PCC are highly condensed, whereas those from late GІ, PCC are long and extended. Morphological assessment of PCC (Table 1) revealed that 62% of GІ, PCC in control cultures were in late GІ. In contrast, only 35% of tamoxifen-treated GІ, PCC were present in late GІ (p = 0.001). The majority (65%) of tamoxifen-treated cells had accumulated early in GІ. Furthermore, exact chromosome counts of GІ, PCC revealed that both control and tamoxifen-treated GІ cells had similar chromosome numbers (70), indi-
DISCUSSION

We have demonstrated that the antiestrogen, tamoxifen, inhibits proliferation of estrogen receptor-positive human breast cancer cells. These results are similar to those reported by Lippman et al. (9) and Sutherland et al. (17) who found that, at concentrations of tamoxifen observed in women treated for breast cancer (≤1.0 μM), the antiestrogen slowed proliferation but was not lethal to MCF-7 cells in short-term culture. At suprapharmacological concentrations (10 μM), a cytocidal effect was observed (17), although the relevance of this effect to in vivo drug mechanism of action is speculative. The slight differences observed in the effects of tamoxifen in these studies may be related to differences in the type (charcoal stripped or not) and concentration of serum used.

Our data suggest that tamoxifen inhibits cell proliferation by invoking a transition delay or block in early to mid-G1 phase of the cell cycle. This delay may require more than one complete passage of some cells through the cell cycle, since cell number more than doubled before plateau growth was observed, and since 72 to 96 hr (2 to 3 generation times) were required for maximal accumulation of cells in G1. A small percentage of cells was refractory to tamoxifen and remained in the proliferative pool (5 to 10% in S phase). The mechanism of resistance of these cells to tamoxifen will require further study. We have shown recently that the tamoxifen effect can be reversed by the addition of 17β-estradiol and that tamoxifen has no effect on the cell cycle kinetics of the receptor-negative MDA-MB-231 cells, suggesting that the antiestrogen effect is mediated through the estrogen receptor (13). Furthermore, these inhibitory effects are not restricted to tamoxifen. In our preliminary studies, the antiestrogens nafloxidine and trioxifene have identical effects on breast cancer cell kinetics.

These data are consistent with the hypothesis that antiestrogens, and perhaps other forms of endocrine therapy, induce regression of breast cancer in vivo by simply blocking progression of estrogen-dependent cells through the cell cycle rather than by a direct cytotoxic effect. With cell replication inhibited, tumors might then regress because of ongoing cell shedding or death or because of interaction with normal host defenses. This hypothesis would account for the very slow rate of tumor regression observed in many patients undergoing endocrine therapy.

These results may have important clinical implications for the treatment of breast cancer. (a) If antiestrogens are simply putting endocrine-responsive breast cancer cells into a "resting" phase of the cell cycle (G0-G1), then treatment of patients after surgery for primary breast cancer with adjuvant antiestrogens may have to be continued indefinitely or at least long enough for host defense or normal cell attrition to eradicate residual tumor cells. Premature discontinuation of therapy would lead to tumor regrowth. (b) The combined use of antiestrogens with cytotoxic chemotherapy in an attempt to obtain an additive or synergistic effect will require careful evaluation. Tamoxifen might enhance the killing effect of drugs most active in G1 cells, whereas the cytotoxic effect of drugs acting specifically during DNA synthesis (S phase) might be diminished. This hypothesis is currently being investigated.

ACKNOWLEDGMENTS

We would like to acknowledge the technical assistance of Philip Estrada, Sharon Olson, and Kathy Mosly.

REFERENCES

Effects of Tamoxifen on Human Breast Cancer Cell Cycle Kinetics: Accumulation of Cells in Early G_1 Phase

C. Kent Osborne, David H. Boldt, Gary M. Clark, et al.


Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/43/8/3583

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.