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Tamoxifen Induces Accumulation of MCF 7 Human Mammary Carcinoma Cells in the G_0/G_1 Phase of the Cell Cycle

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Abstract—*When MCF 7 human mammary carcinoma cells in exponential growth phase were treated with tamoxifen a dose-dependent inhibition of cell growth was observed. This inhibition was accompanied by a dose-dependent decrease in the percentage of S phase cells and a concomitant increase in the percentage of cells in the G_0/G_1 phase of the cell cycle. Simultaneous treatment of cultures with a 10-fold lower concentration of oestradiol completely reversed the growth inhibitory and cell cycle effects of tamoxifen at doses below 5 μM but only partially reversed the effects of higher doses of this drug. It is concluded:* (1) *that tamoxifen-induced growth inhibition is associated with major changes in the cell cycle kinetic parameters of MCF 7 cells, indicating that this drug is a cell cycle phase-specific growth inhibitory agent and* (2) *that not all the anti-proliferative and cell cycle effects of tamoxifen* in vitro *are reversed by simultaneous treatment with oestradiol. This suggests that tamoxifen, in addition to having effects on cell proliferation that are reversed by oestrogens and are likely to be oestrogen receptor-mediated, has antitumour activity* in vitro *that involves biochemical mechanisms independent of the oestrogen receptor system.*

INTRODUCTION

OESTROGENS can induce extensive cellular proliferation in many of their target tissues but the biochemical and cellular kinetic basis of this phenomenon has not been studied extensively [1]. The literature on the effects of oestrogens on cell cycle kinetics has been reviewed recently [2]. It was concluded that oestrogens had three major effects on the cell kinetics of those cells that responded to oestrogen with increased proliferation rates, i.e. they increased the growth fraction by recruiting non-cycling cells into the cell cycle, shortened the overall cell cycle time due mainly to a reduction in the length of G_1 phase and decreased the cell death rate [2]. However, the relative contribution of each of these factors varied with target tissue, e.g. in MCF 7 cells the increased rate of cell proliferation induced by 10^{-9} M oestradiol was attributed solely to a shortening of the cell cycle time [3].

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In recent years the synthetic nonsteroidal antioestrogen tamoxifen has been used effectively in the treatment of oestrogen receptor-positive human breast cancer, but the molecular basis of its antitumour activity has yet to be fully elucidated. Whilst it is known that this and structurally related drugs will inhibit tritiated thymidine incorporation and growth of some human mammary carcinoma cells *in vitro* [4–9], there are no detailed data on the effects of nonsteroidal antioestrogens on the cell cycle kinetic parameters of responsive cells. If anti-oestrogens are antitumour agents solely as a result of their antioestrogenic activity, they are likely to act at the same loci within the cell cycle as oestrogens, i.e. they would be predicted to decrease the growth fraction, lengthen G_1 phase and increase cell death rate.

In this study the effects of oestradiol and tamoxifen on the cell cycle kinetic parameters of the oestrogen receptor (ER)-positive human mammary carcinoma cell line MCF 7 have been investigated using flow cytometry.

MATERIALS AND METHODS

Materials

Tamoxifen (trans 1-(4-β-dimethylaminoeth-oxyphenyl)-1,2-diphenylbut-1-ene) was supplied by ICI Pharmaceuticals Division, Macclesfield, Cheshire, U.K. and oestradiol-17β was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Stock solutions of these drugs were prepared in ethanol and stored at -20°C.

The fluorochromes ethidium bromide and mithramycin were supplied by the Sigma Chemical Co. and Pfizer Inc., New York, NY, U.S.A. respectively. Solutions of these compounds were prepared and stored as previously described [10].

Cell culture

MCF 7 human mammary carcinoma cells in their 299th passage were supplied by Dr Charles McGrath, Meyer L. Prentis Cancer Center, Detroit, MI, U.S.A. and were routinely maintained in RPMI 1640 medium containing 0.34 g/l arginine, 0.63 g/l asparagine and 0.04 g/l folic acid and supplemented with 20 mM HEPES buffer, 14 mM sodium bicarbonate, 5 mM L-glutamine, 20 μg/ml gentamicin, 10 μg/ml insulin and 10% foetal calf serum. Cells in passages 304 or 307 were transferred into the same medium containing 5% foetal calf serum that had been treated with dextran-coated charcoal to remove endogenous steroids. These cells were passaged at weekly intervals in charcoal-treated serum. The stock cells used to set up the experiments described in this paper were from passage Nos 318–329, of which the last 14–22 passages were in medium supplemented with 5% charcoal-treated foetal calf serum. All experiments were conducted in this growth medium.

MCF 7 cells (2×10^5) in exponential growth phase were plated into 25-cm^2 flasks in 5 ml of medium. When cell numbers were approximately 3×10^5 the medium was changed and the drugs (oestradiol and/or tamoxifen) were added from the ethanolic stock solutions such that the final ethanol concentration was 0.1% in all flasks. At various times cells were harvested with 0.05% trypsin and 0.02% EDTA in phosphate-buffered saline, viable cell counts were made under phase-contrast on a haemocytometer and the cells prepared for flow cytometry.

Flow cytometry

MCF 7 cell preparations containing 10^5 chick erythrocytes as an internal standard were stained for DNA flow cytometry with ethidium bromide/mithramycin as previously described [10]. RNase (ribonuclease type 1A, Sigma Chemical Co.) was added directly to the stained cell preparation 5–15 min before analysis to yield a final concentration of 1 mg/ml. Analysis was performed on an ICP 22 pulse cytometer (Ortho Instruments, Westwood, MA, U.S.A.) with excitation at 360–460 nm and fluorescence detected at greater than 550 nm. Estimates of the cell cycle kinetic parameters, i.e. the proportion of cells in the G_0/G_1, S and G_2 + M phases of the cell cycle, were calculated from the resulting DNA histograms using a planimetric method of analysis [11].

RESULTS

The effects of different doses of tamoxifen on the growth of MCF 7 cells in medium supplemented with 5% charcoal-treated foetal calf serum are illustrated in Fig. 1. Control cells grew exponentially, with a mean doubling time of about 27 hr. Tamoxifen caused a dose-dependent decrease in viable cell numbers. Under these experimental conditions 5 μM tamoxifen was the lowest dose of drug that resulted in a significant reduction in cell number after 72 hr of treatment (Fig. 1). When the dose was increased to 10 μM cell numbers remained almost static during the course of the experiment. The two highest doses, 15 and 20 μM, were cytotoxic, inducing rapid decreases in viable cell number (Fig. 1).

When assessed under identical experimental conditions a 1000-fold concentration range (1 nM–1 μM) of oestradiol had no significant effect on viable cell number/flask after 72 hr of treatment (Table 1). When even higher doses of

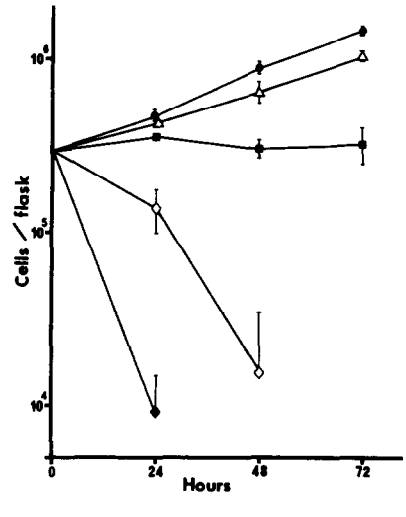

Fig. 1. Effect of tamoxifen on the growth of MCF 7 cells. Exponentially growing cells (2×10^5 in 5 ml of medium containing 5% charcoal-treated foetal calf serum) were plated into 25-cm^2 flasks and 24 hr later the medium was changed and tamoxifen added at concentrations of 0 (•), 5 (△), 10 (■), 15 (◇) or 20 μM (◆). Replicate flasks were harvested 24, 48 and 72 hr after addition of the drug and viable cell counts made. Data points are the mean ± S.E.M. of 3–7 flasks. Where error bars are not shown they did not exceed the size of the symbol.

Table 1. *Effect of tamoxifen and/or oestradiol treatment on viable cell numbers at* 72 hr

Treatment	Cell Nos/flask* ($\times 10^6$)
Control	2.00 ± 0.20
Oestradiol 1 nM	2.10 ± 0.00
10 nM	2.10 ± 0.10
100 nM	2.10 ± 0.10
500 nM	2.10 ± 0.10
1 μM	2.20 ± 0.10
Tamoxifen 5 μM	1.60 ± 0.10
+ 0.5 μM oestradiol	2.10 ± 0.10
Tamoxifen 10 μM	0.73 ± 0.03
+ 1 μM oestradiol	0.53 ± 0.06

*Results are from one experiment and represent the mean ± S.E.M. of 4 flasks per treatment.

oestradiol were tested it was observed that 5 μM was without effect while 10 μM caused a 40% decrease in cell number after 72 hr of treatment (data not shown).

Simultaneous treatment with tamoxifen and a 10-fold lower concentration of oestradiol completely reversed the growth inhibitory effects of tamoxifen administered at 5 μM. However, when 1 μM oestradiol was administered together with 10 μM tamoxifen oestradiol augmented the tamoxifen-induced decrease in viable cell number (Table 1).

Changes in the cell cycle kinetic parameters were also monitored under the same experimental conditions and the data are summarized in Figs 2–4 and Table 2. In Fig. 2 the DNA distribution histograms for control cells and cells that had

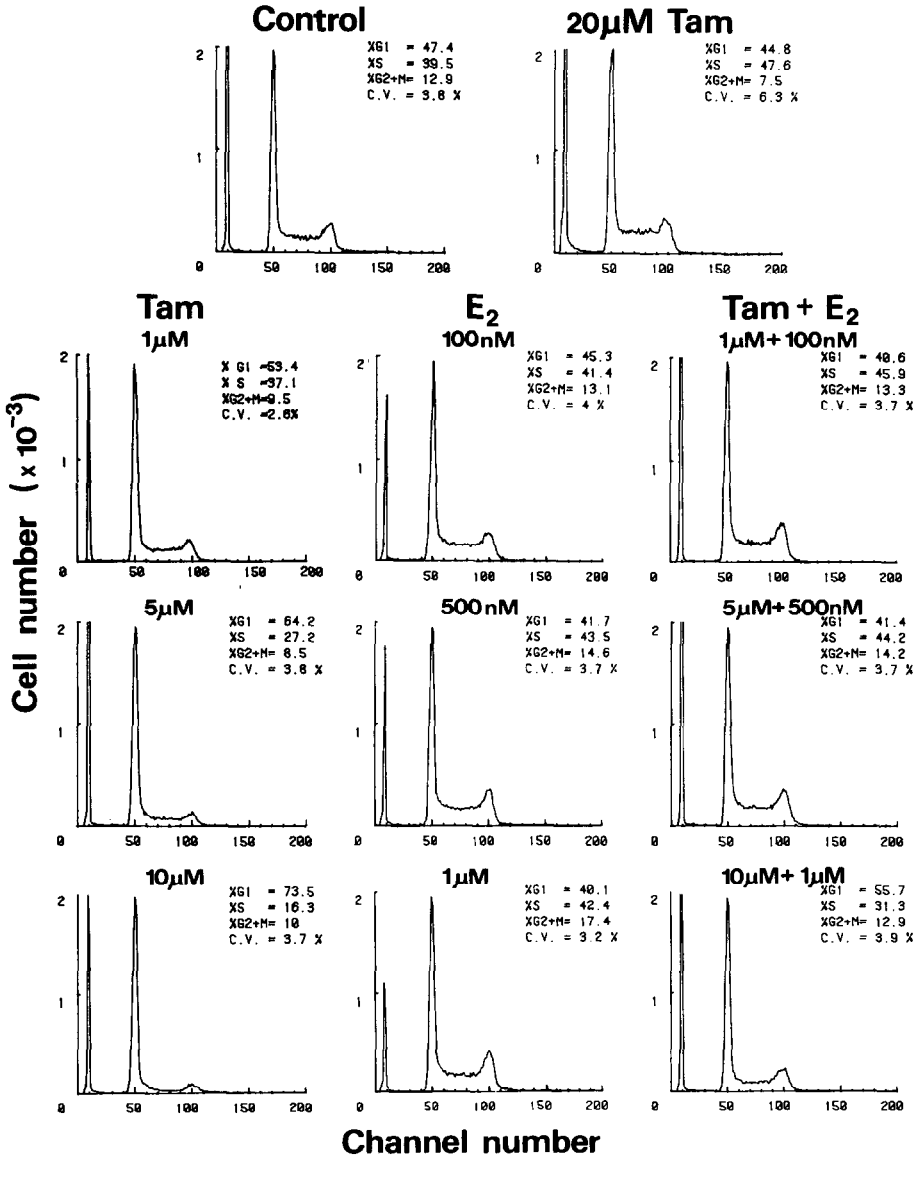

Fig. 2. *Effect of tamoxifen and/or oestradiol on the DNA distribution histograms of MCF* 7 *cells* 36 hr *after drug treatment. The histogram for* 20 μM *tamoxifen represents cells that had been exposed to drug for* 24 hr *only.*

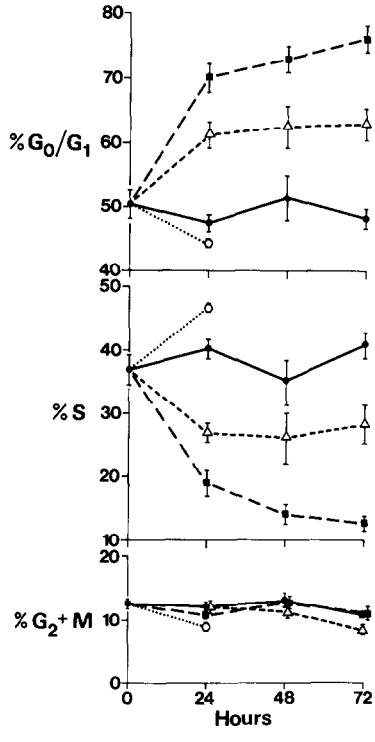

Fig. 3. Effect of tamoxifen on the cell cycle kinetic parameters of MCF 7 cells. The experimental conditions are described in the legend to Fig. 1. The tamoxifen treatments were: control (•), 5 (△), 10 (■) and 20 μM (○). Data points are the mean ± S.E.M. of 5–11 replicate flasks. Too few cells remained after 24 hr exposure to 20 μM tamoxifen to allow accurate estimates of cell cycle kinetic parameters at later time points.

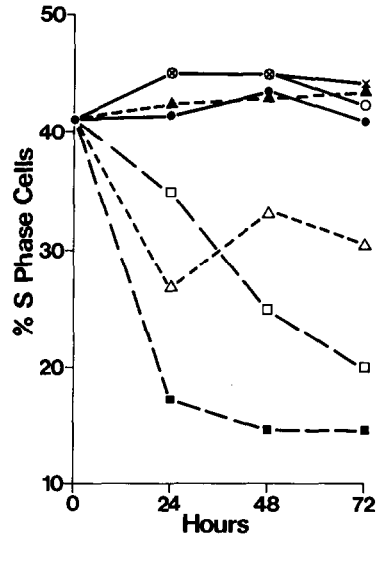

Fig. 4. Effect of tamoxifen and/or oestradiol on the proportion of MCF 7 cells in S phase of the cell cycle. The experimental conditions are described in the legend to Fig. 1. The following drug treatments were used: control (•), 5 μM tamoxifen (△), 10 μM tamoxifen (■), 500 nM oestradiol (○), 1 μM oestradiol (×), 5 μM tamoxifen + 500 nM oestradiol (▲) and 10 μM tamoxifen + 1 μM oestradiol (□). Data are the mean of two flasks.

Table 2. Effect of tamoxifen and/or oestradiol on the percentage of cells in S phase of the cell cycle

Treatment		Proportions of cells in S phase* (% control)
Tamoxifen	1.0 μM	91.1 ± 2.9 (8)
	2.5	83.9 ± 2.0 (9)
	5.0	69.4 ± 1.8 (21)
	7.5	52.1 ± 5.0 (11)
	10.0	39.1 ± 2.1 (21)
	12.5	33.8 ± 4.4 (5)
	15.0	59.2 ± 9.9 (5)
	20.0	112.6 ± 1.8 (5)
Oestradiol	1 nM	110.8 ± 2.6 (5)
	10	112.2 ± 2.7 (5)
	100	110.9 ± 3.3 (5)
	500	109.0 ± 2.1 (8)
	1 μM	109.7 ± 2.8 (8)
Tamoxifen + oestradiol		
1.0 μM	100 nM	118.1 ± 1.1 (5)
2.5	250	115.8 ± 5.4 (5)
5.0	500	104.4 ± 3.2 (8)
7.5	750	75.5 ± 8.9 (5)
10.0	1 μM	63.2 ± 7.0 (7)

*Data are the proportion of cells in S phase expressed as a percentage of control flasks harvested at the same time. Mean ± S.E.M. (number of observations).

been treated with various doses of tamoxifen for 36 hr are shown. The first fluorescent peak represents the chicken red blood cell internal marker [10], while the peak at channel number 50 represents cells with a G_0/G_1 DNA content and the smaller peak at twice that fluorescence, i.e. channel 100, represents cells with a $G_2 + M$ DNA content. Cells in S phase have fluorescence intensities between these limits. Tamoxifen treatment at doses between 1 and 12.5 μM resulted in a dose-dependent decrease in the percentage of S phase cells and a concomitant increase in the percentage of cells in the G_0/G_1 phase (Fig. 2 and Table 2). At 20 μM perturbation in the cell cycle kinetic parameters was less marked, although there was an increase in the percentage of cells in S phase (Fig. 2 and Table 2). This probably indicates that at this high cytotoxic dose tamoxifen kills cells in all phases of the cell cycle with almost equal potency, but with a relative sparing of S phase cells.

When the tamoxifen-induced changes in cell cycle kinetic parameters were monitored at 24-hr intervals during the course of a 72-hr experiment it was apparent that the majority of changes occurred during the first 24 hr of exposure to the drug and thereafter remained relatively constant (Fig. 3). For this reason it was possible to pool the data from the 24-, 36-, 48- and 72-hr time points to

give a more accurate indication of the effects of various doses of the drug on the percentage of S phase cells. These data are summarized in Table 2. Although 5 μM was the lowest dose that caused a significant reduction in cell number at 72 hr, the two lower doses (1 and 2.5 μM) caused a significant reduction in the percentage of S phase cells when compared with the control (Table 2). There was a clear dose-dependent decrease in the percentage of S phase cells between 1 and 12.5 μM, but at the two highest doses (15 and 20 μM) the percentage of S phase cells began to increase again and at the highest dose exceeded that in the control flasks. These latter two doses were also cytotoxic (Fig. 1).

Despite a lack of effect on viable cell numbers at 72 hr, doses of oestradiol between 1 nM and 1 μM caused a significant increase in the percentage of cells in S phase (Fig. 2 and Table 2). The mean increase above the control was about 10% and was not dose-related over the concentration range studied, i.e. 1 nM–1 μM. This may indicate that the effect is maximal by 1 nM. Increasing the dose to 5 μM was without effect, but at 10 μM oestradiol the percentage S phase cells was reduced by about 30%, with a concurrent increase in the percentage G_0/G_1 cells (data not shown).

At doses of tamoxifen between 1 and 10 μM simultaneous treatment with a 10-fold lower dose of oestradiol inhibited the tamoxifen-induced reduction in S phase cells (Fig. 2 and Table 2). This was also apparent at 12.5 and 15 μM, but the synergism of oestradiol and tamoxifen on cell death rate at these doses left insufficient cells for an accurate estimation of the cell cycle kinetic parameters. The ability of a 10-fold lower dose of oestradiol to completely reverse the tamoxifen-induced decrease in S phase cells was dose-related (Fig. 2 and Table 2). At doses of tamoxifen below 5 μM complete reversal, to the level seen in oestrogen-treated cultures, was observed on addition of a 10-fold lower dose of oestradiol, while at higher tamoxifen doses oestradiol could only partially reverse the effects of tamoxifen on the cell cycle kinetic parameters (Fig. 2 and Table 2). Varying the oestradiol concentration over a 1000-fold range did not lead to further reversal of the tamoxifen effect (data not shown).

To investigate further the kinetics of oestrogen-reversibility MCF 7 cells were treated with 5–10 μM tamoxifen in the presence or absence of a 10-fold lower dose of oestradiol and the percentage of S phase cells was monitored for 72 hr. The data are presented in Fig. 4. When tamoxifen was administered at 5 μM the decrease in the percentage of S phase cells was reversed to control levels with 500 nM oestradiol at all time points studied. In contrast, the effects of 10 μM tamoxifen were not completely reversed by 1 μM oestradiol and the degree of reversal induced by oestradiol decreased with the time of exposure to the combination of drugs (Fig. 4). For this reason the data in Table 2 representing the pool of 24- to 72-hr samples for 7.5 and 10 μM tamoxifen plus oestradiol underestimate the long-term effects of this drug combination on the percentage of S phase cells.

DISCUSSION

These data illustrate that tamoxifen and oestradiol have significant effects on the cell cycle kinetic parameters of MCF 7 cells in the exponential growth phase. Three distinct effects of tamoxifen were apparent, i.e. an oestrogen-reversible growth inhibitory effect which was associated with an accumulation of cells in the G_0/G_1 phase of the cell cycle, an oestrogen-irreversible growth inhibitory effect associated with similar kinetic changes and a cytotoxic effect which at the highest dose induced rapid cell death without major changes in cell cycle parameters. It has not yet been possible to establish whether this tamoxifen-induced decrease in cell growth rate is due to a decrease in the rate of cell proliferation, an increase in cell death rate or both. It certainly appears likely that cell death rate is increased under the present *in vitro* conditions at doses above 10 μM.

The dose-responsiveness of these three effects requires some comment in view of the fact that in this study 5 μM was the minimum dose of tamoxifen that caused a significant decrease in cell number at 72 hr, while others have reported significant decreases in MCF 7 cell growth rates in 5% foetal calf serum with doses of 1 μM tamoxifen [8]. When the foetal calf serum concentration was reduced to 1% Coezy *et al.* found significant growth inhibition with 100 nM tamoxifen [12]. Studies in this and other laboratories have shown that the response of human breast cancer cell lines to tamoxifen *in vitro* is strongly influenced by the culture conditions employed [9]. Not only is the response influenced by the drug concentration and time of exposure but also by the concentration of foetal calf serum, the removal of endogenous steroids and other low molecular weight substances by charcoal treatment of the foetal calf serum, the frequency with which the medium and/or drug is replenished and the presence or absence of other hormones and growth factors, e.g. insulin. These variables make it difficult to compare various studies on tamoxifen sensitivity performed under different experimental conditions. However, the observation that under the present assay conditions 1 μM

tamoxifen caused a significant reduction in S phase cells (Table 2) which is likely to be associated with a significant decrease in cell number when cells are exposed for longer time periods suggests that the sensitivity of MCF 7 cells to tamoxifen reported herein is within the range reported by others [4, 9, 12]. This has recently been confirmed in studies where MCF 7 cells were grown in 5% foetal calf serum for 6 days and 100 nM tamoxifen induced significant reductions in the cell number and percentage of S phase cells (Sutherland *et al.*, unpublished observations).

The changes in cell cycle kinetic parameters seen with doses of 1–12.5 μM tamoxifen are compatible with tamoxifen acting at the same loci as oestrogen within the cell cycle [2]. However, similar kinetic changes would be seen with any agent whose only effect was to inhibit the progression of cells through G_1. The dose-dependent decrease in cell growth rate was accompanied by a decrease in the proportion of cells synthesizing DNA (S phase) and a concomitant increase in the percentage of cells in G_0/G_1 phase. In the current experiments it was impossible to distinguish between G_0 and G_1 cells and for this reason it is unclear whether cells accumulate in G_0/G_1 because they are leaving the cell cycle, i.e. entering G_0 phase, or because of a drug-induced lengthening of G_1. It is possible that both mechanisms are operating.

Although oestradiol at doses between 1 nM and 1 μM did not induce a significant increase in cell numbers in these short-term experiments, it did result in a 10% increase in the percentage of cells in S phase, an observation in agreement with previously published effects of oestradiol on MCF 7 cells [3, 5, 13, 14]. If the experiment had been prolonged this increase in S phase cells may well have been accompanied by a significant increase in cell numbers. At a dose of 10 μM oestradiol caused some growth inhibition, but was much less potent than 10 μM tamoxifen (Sutherland *et al.*, unpublished observations). It thus seems unlikely that the effects of high-dose tamoxifen (>10 μM) are due to its oestrogenic properties.

An interesting observation in this study was the inability of oestradiol to completely reverse the effects of tamoxifen at doses where the dose-dependent growth inhibition and S phase depletion were apparent but the nonspecific cytotoxic effect was not yet manifest, i.e. between 5 and 10 μM. Varying the oestradiol concentration over a 1000-fold range did not lead to further reversal of the tamoxifen effect. This observation is compatible with other recently published data which illustrate that antioestrogen treatment is often more inhibitory than oestrogen removal on human mammary carcinoma cell growth both *in vitro* and *in vivo* [7, 15] and provides further evidence for antioestrogen effects that are probably independent of the oestrogen receptor system. Such a conclusion is further supported by our previous observation that tamoxifen can inhibit the growth of the oestrogen receptor-negative BT 20 cell line and that this growth inhibition is accompanied by an accumulation of cells in the G_0/G_1 phase of the cell cycle [16]. Thus the oestrogen-irreversible effects of tamoxifen on breast cancer cells *in vitro* are associated with biochemical events specific to a particular phase of the cell cycle and should not be seen as nonspecific cytotoxicity. The biochemical mechanisms through which tamoxifen induces these effects remain to be elucidated, as do the events that lead to synergism between high doses of tamoxifen (>10 μM) and oestradiol.

In summary, these data illustrate that the synthetic nonsteroidal antioestrogen tamoxifen is a cell cycle phase-specific growth inhibitory agent with both oestrogen-reversible and oestrogen-irreversible components to its molecular mode of action. This warns against interpreting all effects of tamoxifen as oestrogen agonist or antagonist effects and adds support to the recent suggestion that tamoxifen may act through mechanisms independent of the oestrogen receptor [17]. Whether such mechanisms contribute to its antitumour activity *in vivo* requires further study.

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REFERENCES

1. STORMSHAK F, HARRIS JN, GORSKI J. Nuclear estrogen receptor and DNA synthesis. In: O'MALLEY BW, BIRNBAUMER L, eds. *Receptors and Hormone Action*. New York, Academic Press, 1978, Vol. II, 63–81.
2. SUTHERLAND RL, REDDEL RR, GREEN MD. Effects of oestrogens on cell proliferation and cell cycle kinetics. An hypothesis on the cell cycle effects of antioestrogens. *Eur J Cancer Clin Oncol* 1983, **19**, 309–320.
3. WEICHSELBAUM RR, HELLMAN S, PIRO AJ, NOVE JJ, LITTLE JB. Proliferation kinetics of a human breast cancer line *in vitro* following treatment with 17β-estradiol and 1-β-D-arabinofuranosylcytosine. *Cancer Res* 1978, **38**, 2339–2342.

4. LIPPMAN ME, BOLAN G. Oestrogen-responsive human breast cancer in long term tissue culture. *Nature (Lond)* 1975, **256**, 592–593.
5. LIPPMAN ME, BOLAN G, HUFF J. The effects of estrogens and antiestrogens on hormone-responsive human breast cancer in long-term tissue culture. *Cancer Res* 1976, **36**, 4595–4601.
6. ZAVA DT, CHAMNESS GC, HORWITZ KB, MCGUIRE WL. Human breast cancer: biologically active estrogen receptor in the absence of estrogen? *Science* 1977, **196**, 663–664.
7. ALLEGRA JC, LIPPMAN ME. Growth of a human breast cancer cell line in serum-free hormone-supplemented medium. *Cancer Res* 1978, **38**, 3823–3829.
8. HORWITZ KB, KOSEKI Y, MCGUIRE WL. Estrogen control of progesterone receptor in human breast cancer: role of estradiol and antiestrogen. *Endocrinology* 1978, **103**, 1742–1751.
9. BUTLER WB, KELSEY WH, GORAN N. Effects of serum and insulin on the sensitivity of the human breast cancer cell line MCF-7 to estrogen and antiestrogens. *Cancer Res* 1981, **41**, 82–88.
10. TAYLOR IW. A rapid single step staining technique for DNA analysis by flow microfluorimetry. *J Histochem Cytochem* 1980, **28**, 1021–1024.
11. MILTHORPE BK. FMFPAKI: a program package for routine analysis of single parameter flow microfluorimetric data on a low cost minicomputer. *Comput Biomed Res* 1980, **13**, 417–429.
12. COEZY E, BORGNA JL, ROCHEFORT H. Tamoxifen and metabolites in MCF_7 cells: correlation between binding to estrogen receptor and inhibition of cell growth. *Cancer Res* 1982, **42**, 317–323.
13. LIPPMAN ME, MONACO ME, BOLAN G. Effects of estrone, estradiol and estriol on hormone-responsive human breast cancer in long-term tissue culture. *Cancer Res* 1977, **37**, 1901–1907.
14. JOZAN S, MOURE C, GILLOIS M, BAYARD F. Effects of estrone on cell proliferation of a human breast cancer (MCF-7) in long term tissue culture. *J Steroid Biochem* 1979, **10**, 341–342.
15. SHAFIE SM, GRANTHAM FH. Role of hormones in the growth and regression of human breast cancer cells (MCF 7) transplanted into athymic nude mice. *J Natl Cancer Inst* 1981, **67**, 51–56.
16. GREEN MD, WHYBOURNE AM, TAYLOR IW, SUTHERLAND RL. Effects of antioestrogens on the growth and cell cycle kinetics of cultured human mammary carcinoma cells. In: SUTHERLAND RL, JORDAN VC, eds. *Non-steroidal Antioestrogens*. Sydney, Academic Press, 1981, 319–412.
17. BLACK LJ, GOODE RL. Evidence for biological action of the antiestrogens LY 11708 and tamoxifen by different mechanisms. *Endocrinology* 1981, **109**, 987–989.