Idoxifene Antagonizes Estradiol-dependent MCF-7 Breast Cancer Xenograft Growth through Sustained Induction of Apoptosis

Stephen R. D. Johnston,1 Irene M. Boedinghaus, Sharon Riddler, Ben P. Haynes, Ian R. Hardcastle, Martin Rowlands, Rachel Grimshaw, Michael Jarman, and Mitch Dowsett


ABSTRACT

Idoxifene is a novel selective estrogen (E2) receptor (ER) modulator that is currently in clinical development for the treatment of breast cancer. Compared to tamoxifen, idoxifene is metabolically more stable, with a higher relative binding affinity for the ER and reduced agonist activity on breast and uterine cells. Idoxifene also inhibits calmodulin, a calcium-binding protein that is involved in cell signal transduction pathways.

In this study, the abilities of idoxifene and tamoxifen to antagonize E2-dependent MCF-7 xenograft growth in oophorectomized athymic mice were compared. The basis for idoxifene’s antitumor activity was examined by comparing the effectiveness of the clinically used trans-isomer (referred to here as idoxifene) with its cis-isomer, which has a 50-fold lower relative binding affinity for ER than idoxifene but similar calmodulin-inhibitory activity. Changes in tumor cell proliferation, apoptosis, and ER-dependent protein expression were studied. Both idoxifene and tamoxifen significantly inhibited E2-dependent tumor growth, whereas cis-idoxifene had little effect. Withdrawal of E2 support induced significant tumor regression due to impaired cell proliferation (Ki-67 score, 9 versus 51% compared to E2 controls) and induction of apoptosis (3.6 versus 0.9% compared to E2 controls). Both anti-E2s inhibited cell proliferation and caused a significant 3-fold induction of apoptosis in E2 supported tumors after 1 week, which was maintained for 3 months with idoxifene (3.1 versus 0.48% compared to E2 controls) but decreased back to baseline in tumors treated with tamoxifen (0.69%). In contrast, cis-idoxifene had no effect on either cell proliferation or apoptosis. Both tamoxifen and idoxifene initially induced ER expression, whereas prolonged therapy with tamoxifen significantly reduced progesterone receptor levels.

In conclusion, idoxifene resulted in similar inhibition of E2-dependent MCF-7 xenograft growth compared with tamoxifen, an effect that is mediated via ER rather than through calmodulin. Sustained induction of apoptosis may contribute to prolonged antagonism of E2-dependent growth, and it occurred to a greater extent following 3 months of idoxifene, compared to tamoxifen.

INTRODUCTION

Idoxifene is a novel selective ER2 modulator that is structurally related to tamoxifen (1). Analogues of tamoxifen, which include an iodine atom at position 4, have increased affinity for the ER (2), and studies have confirmed that idoxifene is metabolically more stable than tamoxifen (3, 4). Idoxifene inhibits hormone-dependent breast cancer growth and is more effective than tamoxifen at inhibiting both MCF-7 cell growth in vitro and rat mammary tumor growth in vivo (5). Its reduced agonist activity in the immature rat uterotrophic assay (6) may account for its more favorable profile of activity when compared with tamoxifen. Anti-E2s inhibited cell proliferation and caused a significant 3-fold induction of apoptosis in E2 supported tumors after 1 week, which was maintained for 3 months with idoxifene (3.1 versus 0.48% compared to E2 controls) but decreased back to baseline in tumors treated with tamoxifen (0.69%). In contrast, cis-idoxifene had no effect on either cell proliferation or apoptosis. Both tamoxifen and idoxifene initially induced ER expression, whereas prolonged therapy with tamoxifen significantly reduced progesterone receptor levels.

In conclusion, idoxifene resulted in similar inhibition of E2-dependent MCF-7 xenograft growth compared with tamoxifen, an effect that is mediated via ER rather than through calmodulin. Sustained induction of apoptosis may contribute to prolonged antagonism of E2-dependent growth, and it occurred to a greater extent following 3 months of idoxifene, compared to tamoxifen.

MATERIALS AND METHODS

Chemicals. The two anti-E2s used in this study were tamoxifen \{\{Z-trans-1-[4-(2-dimethylamino)ethoxy]phenyl]-1,2-diphenyl-1-butenec\}\} from Sigma Chemical Co. (Poole, United Kingdom) and idoxifene \{E-trans-1-(4-iodophenyl]-1-[4-(2-pyridylidino)ethoxy]phenyl]-2-phenyl-1-butenec\} synthesized at The Cancer Research Campaign Centre for Cancer Therapeutics, Institute of Cancer Research (Sutton, United Kingdom), and cis-idoxifene (CB 7709), which was synthesized as follows. A mixture of Z-1-(4-(2-chlorothiophenyl]-1-(4-iodophenyl]2-phenyl-1-butenec (0.498 g, 1 mmol; synthesis of this intermediate was described in Ref 2), pyridoline (2 ml), and ethanol (10 ml) was refluxed for 16 h. The mixture was concentrated in vacuo, and the residue was dissolved in ether and washed with sodium bicarbonate solution (20 ml) and water (20 ml). The organic layer was dried (Na2SO4) and concentrated in vacuo. The residues were separated by flash chromatography (Silica 15111; Merck, Leicester, United Kingdom; ether eluent) to give the title compound (0.37 g, 71%). Recrystallization from ethanol gave white crystals (0.26 g, melting point of 103–105°C).

Drug Delivery System. Slow-release silastic capsules of tamoxifen, idoxifene, and cis-idoxifene were made according to previously published methods (11). Briefly, these were formed by plugging one end of a 1.5-cm length of medical-grade silastic tubing (0.078-inch internal diameter by 0.125-inch outside diameter; Dow Corning, Midland, MI) with Silastic 382 medical-grade adhesive. After drying, these were filled with either tamoxifen free base or the crystalline form of idoxifene or cis-idoxifene. On the basis of the relative anti-E2 in circumstances when tamoxifen’s agonist activity is predominant (5). We recently demonstrated that, in the absence of estradiol, idoxifene inhibited the growth of MCF-7 xenografts significantly more than tamoxifen, which was associated with a reduced frequency of tumors with acquired anti-E2 resistance (6).

In this study, we compared the abilities of idoxifene and tamoxifen to antagonize E2-dependent growth in the same MCF-7 xenograft model system. In the first part of the experiment, we examined idoxifene’s effect on growth in relation to its ability to bind ER and antagonize the calcium-binding protein calmodulin. Calmodulin plays a key role in several processes that govern cell proliferation, and tamoxifen is known to antagonize the formation of a complex between calmodulin and ER (7). Analogues of tamoxifen, including a 4-iodo substitution, idoxifene, have been found to be more potent inhibitors of calmodulin function in vitro than tamoxifen (8, 9), and cytotoxicity of tamoxifen analogues against MCF-7 cells using the short-term MT assay in vitro has been correlated with their calmodulin antagonism (10). The chemically synthesized cis-isomer of idoxifene (Fig 1) has a 50-fold lower RBA for ER than for the clinically used trans-isomer of idoxifene, but it remains a potent inhibitor of calmodulin-dependent cAMP phosphodiesterase (Table 1). By comparing the two different isomers, we anticipated that the relative contribution of calmodulin inhibition and ER antagonism to idoxifene’s growth inhibition could be determined in vivo.

In the second part of the study, we compared the effects of idoxifene, cis-idoxifene, and tamoxifen on proliferation (as measured by Ki-67 staining) and apoptosis (as measured by the TUNEL assay) in the harvested tumor. In addition, we measured tumor expression of the E2-dependent proteins ER and PgR and recorded uterine wet weights at the end of the experiment as indicators of each drug’s antagonism of estradiol’s effects on breast and uterine tissue, respectively.

Received 12/22/98; accepted 6/3/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom requests for reprints should be addressed, at Department of Medicine, Royal Marsden NHS Trust, Fulham Road, London, SW3 6JJ, England. Phone: 0171-352-8171 ext. 2748; Fax: 0171-352-5441; E-mail: stephen@icr.ac.uk.

2 The abbreviations used are: ER, estrogen receptor; E2, 17β-estradiol/estrogen; RBA, relative binding affinity; PgR, progesterone receptor; TUNEL, terminal deoxynucleotidyl transferase-mediated nick end labeling; OHT, 4-hydroxytamoxifen; DMT, N-desmethyl-tamoxifen; DDIT, di-desmethyltamoxifen.

The Institute of Cancer Research, Belmont, Sutton, Surrey, SM2 5NG, England
release pellet plus a placebo silastic capsule; (b) E2 support plus tamoxifen delivered by silastic capsule; (c) E2 support plus idoxifene delivered by silastic capsule; (d) E2 support plus cis-iodixifene delivered by silastic capsule; and (e) withdrawal of E2 support plus placebo silastic capsule. All E2 pellets and drug capsules were inserted under general anesthesia and changed after 8 weeks.

Following 1 week of therapy, five mice from each group were sacrificed to establish the serum levels and biological effects on the tumor at this early time point. The remaining animals continued treatment, and tumor volume was recorded weekly. In both the tamoxifen and idoxifene groups, four mice from each group were sacrificed after 4 weeks of therapy (week 8) due to local reactions with the silastic drug capsule. Although this was not planned in the original experimental design, this gave us an opportunity to examine serum levels and biological effects at this intermediate time point in these two treatment groups. After 12 weeks (3 months) of treatment, the experiment was terminated, animals were sacrificed, and tumor, uterus, and serum were harvested as described above.

Immunohistochemistry for Ki-67 (MIB1), Apoptosis (TUNEL), ER, and PgR. General reagents were purchased from Sigma (Dorset, United Kingdom), and unless otherwise specified, primary antibodies and avidin-biotin complex were from DAKO (Cambridge, United Kingdom). Sections of 3 μm were cut from the formalin-fixed paraffin wax-embedded MCF-7 tumor xenografts and placed on 3-aminopropyltriethoxysilane-coated slides.

For each analytic, sections were stained in one batch together with known positive controls. With the exception of apoptosis, the basic staining procedure was as follows. The sections were dewaxed in xylene and gradually rehydrated to water, and endogenous peroxidase activity was blocked. Antigen retrieval was performed by means of microwaving at 750 W in citrate buffer (pH 6.0) for 10 min, after which buffered sections were cooled to room temperature, and a blocking antibody was applied in PBS (pH 7.4) prior to primary antibody. The sections were incubated for 45 min in 1:200 biotinylated antinouse immunoglobulins and rinsed, and avidin-biotin complex (DAKO) was applied for half an hour. After washing in PBS, the peroxidase-antiperoxidase reaction was developed to a brown stain by 0.05% diaminobenzidene enhanced with 0.07% imidazole and hydrogen peroxide. Cell cytoplasm was counterstained blue with Mayer’s hematoxylin, and the sections were dehydrated, cleared in xylene, and permanently mounted in DePex.

For ER, the 1DS primary antibody (13) at a dilution of 1:100 was incubated for 2 h according to previous validated conditions (14). For the detection of PgR, the sections were incubated for 1 h with the NovoCastra NCL-PgR antibody (Vector Laboratories, Peterborough, United Kingdom) at a 1:30 dilution. MIB1 antibody (The Binding Site Ltd., Birmingham, United Kingdom) was used at a dilution of 1:50 for 1 h for Ki-67 immunostaining. The TUNEL assay was used to identify apoptotic cells in the xenograft tumor sections (15). All sections were examined under a standard light microscope using ×40 objective and 10 × 10 eyepiece incorporating a graticule. The brown nuclear staining resulting from ER and PgR immunostaining was quantified by quickscore (range, 0–18), a scoring system that we have validated against the H-score (16). Ki-67-positive nuclear staining cells were recorded as percentages. The apoptotic index was expressed as a percentage that was calculated from the number of brown staining cells displaying apoptotic bodies of 3000 tumor cells per section counted under high power, excluding any necrotic areas.

Statistics. Growth rates for individual tumors following treatment at week 4 were calculated from the regression coefficient of log (volume) versus time, under assumption of constant growth. This assumption was tested by comparing the growth in weeks 4–7 with that in weeks 8+ using the Wilcoxon signed ranks test, and no evidence of a difference was found. The growth rates were compared between the five groups using the Kruskal-Wallis ANOVA, and between-group comparisons were then undertaken using the Mann-Whitney U test. Comparisons in uterine wet weight versus E2 controls were made by nonparametric Mann-Whitney U test. For the analysis of Ki-67, apoptosis, ER and PgR, nonparametric comparisons (Mann-Whitney U test) were made both at 1 week and 3 months for each treatment group compared with their respective E2 control. In addition, for each treatment group, comparisons were made across different time points with the baseline E2 control tumors. The immunohistochemical data were expressed for each group and time point as the arithmetic mean ± SE.

### Table 1 Comparison of calmodulin antagonism and ER-binding affinity

<table>
<thead>
<tr>
<th></th>
<th>Calmodulin-dependent cAMP/PDE IC₅₀</th>
<th>Rat uterine ER (RBA of E₂ = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamoxifen</td>
<td>6.8 ± 1.1</td>
<td>5.0</td>
</tr>
<tr>
<td>Idoxifene</td>
<td>1.4 ± 0.1</td>
<td>12.0</td>
</tr>
<tr>
<td>cis-Iodoxifene</td>
<td>1.5 ± 0.2</td>
<td>0.25</td>
</tr>
</tbody>
</table>
RESULTS

Tumor Growth. The tumor take rate after implantation was between 89 and 100%, and following 4 weeks of E2 support, the mean tumor sizes and animal weights were very similar among the five randomized groups. Tumor growth continued in control animals that received E2 support alone and had a placebo capsule inserted. In contrast, in controls that had E2 support withdrawn and that also had a placebo capsule inserted, highly significant ($P < 0.0001$) and stable tumor regression was observed (Fig. 2). In three of the animals in whom E2 was withdrawn, no palpable tumor remained at the end of the experiment.

Both tamoxifen and idoxifene significantly inhibited E2-dependent growth for the duration of the experiment (Fig. 2; $P < 0.001$ versus E2 controls). There was no significant difference between the growth rate of the tamoxifen and idoxifene curves ($P = 0.1$), and tumor volumes remained static. In contrast, cis-idoxifene caused minor antagonism of E2-dependent growth, which was not significantly different from E2 controls.

Serum Anti-E2 Levels. After 1 week of therapy, the serum levels of idoxifene and tamoxifen were identical (8.2 ± 2.1 and 8.5 ± 1.2 ng/mL, respectively; Table 2). Prolonged therapy using the silastic capsules resulted in a mean tamoxifen level of 26.6 ng/mL and a mean idoxifene level of 14.0 ng/mL. This was equivalent to steady-state concentrations of 71 ng/mL tamoxifen and 28 ng/mL idoxifene. Metabolite levels for tamoxifen and idoxifene were not measured. cis-Idoxifene gave much higher concentrations of 84.1 ng/mL at week 1 and 95.4 ng/mL after prolonged therapy (Table 2).

Uterine Weights. The mean uterine wet weights in the five treated groups are shown in Table 3. There was no significant difference in uterine weight between E2-treated animals and those who received cis-idoxifene. Animals treated with E2 plus either tamoxifen or idoxifene had significantly smaller uterine weights than did those treated with E2 alone, although those that had E2 therapy withdrawn had the lowest values, which were significantly lower ($P < 0.0001$) than controls. There was no significant difference between the idoxifene- and tamoxifen-treated animals.

Cell Proliferation (Ki-67). Control tumors that were stimulated with E2 and in a rapid growth phase had mean Ki-67 scores of 51.0 ± 2.1%. After 1 week of therapy with tamoxifen plus continued E2 support, there was a reduction in mean Ki-67 score to 24.7 ± 5.8% ($P = 0.047$ versus E2 control at week 1; Fig. 3A). Following 1 week of therapy with idoxifene plus E2, however, there was no significant change in Ki-67 (43.2 ± 5.3%). Likewise, cis-idoxifene-treated tumors had a similar score (43.8 ± 5.4%), whereas maximal reduction in cell proliferation at week 1 was seen after withdrawal of E2, with a highly significant fall in Ki-67 to 9.0 ± 2.3% ($P = 0.009$; Fig. 3A).

In the small series of tumors harvested after 4 weeks of anti-E2 therapy, there was no significant difference between the Ki-67 scores of tamoxifen- and idoxifene-treated tumors (35.2 ± 6.1% and 32.5 ± 7.4%, respectively; $P = 0.77$; data not shown in Fig. 3A). However, these values were both significantly less than E2-treated controls at the start of the experiment ($P = 0.027$). Following prolonged therapy for 3 months, there was no statistical difference in Ki-67 score among E2-stimulated tumors (30.8 ± 2.1%), tamoxifen-treated tumors (38.4 ± 6.0%), and idoxifene-treated tumors (33.3 ± 1.9%). Tumors treated by withdrawal of E2 continued to have the lowest rate of cell proliferation, which was maintained until the end of the experiment (11.7 ± 4.2%; Fig. 3A).

Apoptosis Index. The mean percentage of cells detected by TUNEL (apoptosis index) in E2-treated control tumors was low, at 0.89 ± 0.01%. Following therapy of E2-stimulated tumors for only 1 week with either tamoxifen or idoxifene, there was a significant increase in apoptosis scores compared with tumors treated with E2 alone (tamoxifen, 2.48 ± 0.11%; $P = 0.009$; idoxifene, 2.53 ± 0.08%; $P = 0.009$; Fig. 3B). This was maintained in the small series of tumors analyzed after 4 weeks (1.38 ± 0.44%; tamoxifen; 2.58 ± 0.2%; idoxifene). For tumors treated with cis-idoxifene, no induction of apoptosis was seen at week 1, and values were similar to tumors treated with E2 alone. Maximal induction of apoptosis at week 1 was seen after withdrawal of E2 (3.65 ± 0.24%; Fig. 3B).

By the end of the experiment, after 3 months of therapy, apoptosis in tumors treated with tamoxifen was reduced back to baseline levels (0.69 ± 0.1%; Fig. 3B) and was only marginally higher than those treated with prolonged E2 alone. In marked contrast, in tumors that continued to receive idoxifene plus E2, high levels of apoptosis remained (3.09 ± 0.24%), which were significantly greater than tamoxifen ($P = 0.009$). cis-Idoxifene still showed no induction of apoptosis (Fig. 3B), whereas levels of apoptosis remained highest in tumors deprived of E2 (3.85 ± 0.21%).

ER and PgR. In tumors treated with E2 alone, the mean quick-score for ER during the experiment ranged from 3.6 ± 0.2 to 2.5 ± 0.6 (Table 4). Following the addition of tamoxifen for 1 week to E2-stimulated tumors, there was induction of ER expression to 9.2 ± 1.9 ($P = 0.047$). By the end of the experiment, ER values in tamoxifen-treated tumors had fallen back to baseline and were no longer any different from E2-stimulated tumors or those in which E2 support had been withdrawn (see Table 4). Idoxifene also resulted in an initial induction of ER expression, and at the end of the experiment, the levels of ER in idoxifene-treated tumors were similar to those in tamoxifen-treated tumors. cis-Idoxifene had no effect on ER expression (Table 4).

Maximal PgR expression was seen in E2-stimulated tumors (Table 5). The addition of tamoxifen to E2-stimulated tumors caused a reduction in PgR expression (5.4 ± 1.7; $P = 0.06$), although this was not as great as that observed after withdrawal of E2, where in most tumors, PgR expression was absent (Table 5). Idoxifene caused a slower and less marked reduction in PgR expression, although after 4 weeks of therapy, the PgR values were not significantly different from tamoxifen-treated tumors (Table 5). cis-Idoxifene had no significant effect on PgR expression, and values were similar to those of E2-treated controls.

DISCUSSION

The primary aim of this study was to examine the antagonism of E2-dependent MCF-7 xenograft growth induced by idoxifene in comparison with tamoxifen. This MCF-7 xenograft model retained its hormonal sensitivity, as demonstrated by the exponential growth with E2, together with the almost complete regression of tumors upon withdrawal of E2 support. Both anti-E2s significantly inhibited E2-dependent growth in this model (Fig. 2). The serum levels of tamoxifen and idoxifene were very similar after 1 week (Table 2), and prolonged therapy resulted in a mean concentrations of 71 and 28 nM, respectively, values that are in the same range as that which we published previously using the silastic capsulare delivery system (6).

The chemically synthesized cis-idoxifene isomer, which has a 50-fold lower affinity for ER yet remains as potent an inhibitor of calmodulin (Table 1), was dramatically less effective at inhibiting E2-dependent growth (Fig. 2). This would imply that the growth-inhibitory effect of idoxifene is mediated predominantly through antagonism of the ER rather than any significant effect on calmodulin-dependent signal transduction pathways, which had been suggested by previous in vitro studies (9). There is no evidence that isomerization of trans-idoxifene to its cis-isomer occurs in vivo. Indeed, the iodine at position 4 blocks a major route of metabolism via hydroxylation, which, for tamoxifen, is a prerequisite to isomerization of the potent.

3648
4OHT metabolite to its cis-isomer, which is known to be a weaker ER antagonist, with some agonist properties (17). Osborne et al. (18) first suggested that increased relative levels of cis-4OHT may account for acquired tamoxifen resistance both in MCF-7 xenografts and breast cancer patients (19). However, the role of cis-isomerization in resistance was questioned by Wolf et al. (20), who demonstrated that fixed-ring derivatives of tamoxifen that cannot undergo isomerization were still able to stimulate the growth of tamoxifen-resistant MCF-7 xenografts. Although our xenograft study used the chemically synthesized cis-isomer and confirmed that it is a weak ER antagonist in vivo, the structure of trans-idoxifene limits isomerization, and none have been detected in ongoing clinical studies (21).

For tamoxifen, it is recognized that its metabolites may contribute to the antiestrogenic action of the drug in vivo, although there are clear differences between rodents and humans. Although 4OHT is a potent metabolite with a 20-fold higher RBA for ER than tamoxifen, in humans, serum levels are <1% of the parent compound due to rapid elimination in the liver via glucuronidation. The major route of tamoxifen metabolism in humans is deamination to give DMT and DDMT, which, at steady state, exist at ~150% and 30% of parent compound levels, respectively (22, 23). The DMT and DDMT metabolites have a lower RBA for ER than tamoxifen but, due to their levels, probably contribute to the antiestrogenic activity of the drug as a whole. In rodents, the relative levels of 4OHT are higher and those of DMT/DDMT are somewhat lower than in humans, and these may differ substantially between reports depending on the drug delivery system used (24). In particular, the silastic capsules are reported to have much lower metabolite levels compared with oral or s.c. dosing.

Idoxifene is metabolically more stable than tamoxifen due to the iodine at position 4 that blocks hydroxylation and the pyrrolidino ring, which prevents the N-demethylation seen with tamoxifen (3). In humans, this results in a longer terminal half-life for the parent compound (25). The major metabolites detected in humans include the pyrrolidone, hydroxyethyl, and aminoethyl derivatives, which all retain an intact iodine and represent products of side chain metabolism. These metabolites have RBA values lower than those of idoxifene but equivalent to those of tamoxifen (21). Plasma levels in patients are 31, 88, and 14% those of idoxifene, respectively, such that they probably contribute to idoxifene’s antiestrogenic activity in vivo (21). As with tamoxifen, the metabolism of idoxifene differs in rodents to humans with the 4’-hydroxy metabolite being the major compound detected (4). This metabolite retains the iodine at position 4 with hydroxylation occurring on the opposite phenyl ring and has a slightly higher RBA than idoxifene (26). Although we have detected this metabolite previously in mice given a slow-release s.c. idoxifene pellet, we did not find significant levels in our current experiments using silastic capsules. Although the sensitivity of our high-pressure liquid chromatography was considerably less for this metabolite than for idoxifene pelleted, it is possible that, as with tamoxifen, circulating metabolite levels are somewhat lower when the silastic capsule drug delivery system is used (24).

Uterine wet weight is another surrogate for the effect of anti-E2s on E2-dependent tissues. As with tumor growth, both idoxifene and tamoxifen significantly reduced uterine weight compared with E2-treated controls, whereas cis-idoxifene had no effect and maximal reduction was seen upon total withdrawal of E2 (Table 3). These results suggest that both idoxifene and tamoxifen are associated with equivalent antagonism of E2’s effect on uterine tissue. However, our model was not designed to address the relative agonist activity of each anti-E2 on uterine tissue. In the absence of E2, tamoxifen is a partial agonist on the uterus and will promote the growth of endometrial cancer xenografts in athymic nude mice while, at the same time, inhibiting contralateral MCF-7 breast cancer xenografts (27). It was reported previously that idoxifene has significantly less agonist activity on the uterus than tamoxifen in the absence of E2, using the more sensitive immature rat uterotropic assay (5).

The regression of a tumor after E2 deprivation has been thought to be primarily due to reduced cell proliferation, although it has been recognized that induction of apoptosis is also involved (28). In previous xenograft experiments, we showed a 3-fold induction of apoptosis (TUNEL assay) after 14 days of E2 withdrawal, which was accompanied by slow-release silastic capsule delivery.

### Table 2: Mean serum concentration of tamoxifen, idoxifene, and cis-idoxifene delivered by slow-release silastic capsule

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1-week treatment</th>
<th>12-week treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamoxifen</td>
<td>8.2 ± 2.1</td>
<td>26.6 ± 7.4</td>
</tr>
<tr>
<td>Idoxifene</td>
<td>8.5 ± 1.2</td>
<td>14.0 ± 3.6</td>
</tr>
<tr>
<td>cis-Idoxifene</td>
<td>84.1 ± 13.0</td>
<td>95.4 ± 14.5</td>
</tr>
</tbody>
</table>

*Capsules were replaced after 8 weeks, or sooner if became displaced. Values are expressed as mean ± SE.

### Table 3: Effect of tamoxifen, idoxifene, and cis-idoxifene on uterine wet weight in E2-treated oophorectomized athymic mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Mean uterine wet weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2</td>
<td>15</td>
<td>181.7 ± 9.6</td>
</tr>
<tr>
<td>E2 + tamoxifen</td>
<td>17</td>
<td>109.1 ± 13.2</td>
</tr>
<tr>
<td>E2 + idoxifene</td>
<td>11</td>
<td>134.3 ± 11.9</td>
</tr>
<tr>
<td>E2 + cis-idoxifene</td>
<td>14</td>
<td>187.8 ± 14.5</td>
</tr>
<tr>
<td>Withdrawal of E2</td>
<td>15</td>
<td>35.8 ± 4.6*</td>
</tr>
</tbody>
</table>

Terms: *P < 0.01 vs. E2-treated controls (Mann-Whitney U test).

---

3 S. R. D. Johnston, B. P. Haynes, and M. Dowsett, unpublished data.
by a 75% reduction in cell proliferation (Ki-67 scores; 29). We demonstrated that these changes occur very early, within 3 days, and probably precede significant reduction in tumor volumes. A novel finding from these current experiments is that, even after 3 months of prolonged E2 deprivation, apoptosis remained significantly elevated (Fig. 3B). Thus, a sustained response to E2 deprivation in hormone-sensitive breast cancer xenografts results from both continued cell death and impaired ability of the cells to proliferate. It may be noted that, even over the last few weeks of the experiment, E2-deprived tumors continued to regress, which is consistent with these measurements of Ki-67 and apoptosis continuing to lead to an overall loss of cells.

Until recently, the extent to which antagonism of E2’s action by nonsteroidal anti-E2s may produce the same sustained effect on tumor cell proliferation and apoptosis has been unclear. Both tamoxifen and idoxifene induced apoptosis within the first week (Fig. 3B), in association with an immediate effect on tumor growth (Fig. 2). This supports previous publications of apoptosis in breast cancer cells and xenografts treated with toremifene (30). The induction of apoptosis was maintained after 4 weeks therapy, although following 3 months of tamoxifen administration in this MCF-7 xenograft model is associated with tamoxifen-stimulated tumor regrowth (6, 31, 32). It is possible that the return to baseline in apoptosis and Ki-67 scores that we observed in E2-stimulated tumors following 3 months tamoxifen may reflect early tumor regrowth manifest as changes in these biological parameters of growth.

In contrast, idoxifene’s initial effect on apoptosis was maintained during 3 months of treatment (Fig. 3B), whereas the chemically synthesized cis-idoxxifene had no effect on either apoptosis or proliferation, consistent with cis-idoxxifene’s inability to antagonize E2-dependent growth. The degree of induction of apoptosis with idoxifene approached that observed after long-term withdrawal of E2, whereas the lesser effect on cell proliferation would be consistent with stable tumor volumes rather than continued regression seen with E2 withdrawal. Caution should be used in the interpretation of these differences in apoptosis at 3 months because this was not the primary aim of this study. A more detailed comparative study of harvested tumors at multiple earlier time points (i.e., 3, 7, 14, 28, and 56 days) in conjunction with pharmacokinetic measurements would be required to determine any differential ability of idoxifene to influence apoptosis compared with tamoxifen. Equally, no inference can be made in

Table 4 Mean tumor ER quickscores ± SE in E2-stimulated MCF-7 xenografts after treatment with tamoxifen, idoxifene, or cis-idoxxifene or following withdrawal of E2

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Baseline</th>
<th>1 week</th>
<th>4 weeks</th>
<th>12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2</td>
<td>3.6 ± 0.2</td>
<td>2.8 ± 0.4</td>
<td>2.5 ± 0.6</td>
<td>2.5 ± 0.6</td>
</tr>
<tr>
<td>Tamoxifen + E2</td>
<td>9.2 ± 1.9a</td>
<td>2.5 ± 0.6</td>
<td>3.0 ± 0.5</td>
<td>3.0 ± 0.5</td>
</tr>
<tr>
<td>Idoxxifene + E2</td>
<td>4.6 ± 0.6</td>
<td>5.5 ± 0.1</td>
<td>2.4 ± 0.7</td>
<td>2.4 ± 0.7</td>
</tr>
<tr>
<td>cis-Iodoxxifene + E2</td>
<td>3.5 ± 0.9</td>
<td>5.0 ± 0.6</td>
<td>3.4 ± 0.6</td>
<td>3.4 ± 0.6</td>
</tr>
<tr>
<td>Withdrawal of E2</td>
<td>7.2 ± 0.5a</td>
<td>5.0 ± 0.5</td>
<td>4.0 ± 1.0</td>
<td>4.0 ± 1.0</td>
</tr>
</tbody>
</table>

a The numbers of tumors per group were: n = 5 at baseline and 1 week, n = 4 at week 4, and n = 5–8 after 12 weeks of treatment.
b P < 0.05 vs. E2 control (Mann-Whitney U test).
c P < 0.01 vs. E2 control (Mann-Whitney U test).
Table 5 Mean tumor PgR quickscorers ± SE in E2-stimulated MCF-7 xenografts after treatment with tamoxifen, idoxifene, or cis-idoxifene, or following withdrawal of E2*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline</th>
<th>1 week</th>
<th>4 weeks</th>
<th>12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2</td>
<td>16.8 ± 0.7</td>
<td>11.0 ± 1.2</td>
<td>12.3 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>Tamoxifen + E2</td>
<td>5.4 ± 1.7</td>
<td>6.3 ± 1.0</td>
<td>2.0 ± 0.6b</td>
<td></td>
</tr>
<tr>
<td>Idoxifene + E2</td>
<td>11.2 ± 1.1</td>
<td>9.5 ± 1.9</td>
<td>7.8 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Cis-Idoxifene + E2</td>
<td>11.5 ± 2.4</td>
<td>8.4 ± 1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Withdrawal of E2</td>
<td>0.4 ± 0.2a</td>
<td>1.5 ± 0.5a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The number of tumors per group were: n = 5 at baseline and 1 week, n = 4 at week 4, and n = 5–8 after 12 weeks of treatment.


Idoxifene Antagonizes Estradiol-dependent MCF-7 Breast Cancer Xenograft Growth through Sustained Induction of Apoptosis


Updated version Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/59/15/3646

Cited articles This article cites 37 articles, 15 of which you can access for free at:
http://cancerres.aacrjournals.org/content/59/15/3646.full#ref-list-1

Citing articles This article has been cited by 3 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/59/15/3646.full#related-urls

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.