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Antiestrogen, Trans-Tamoxifen Modulation of Human Breast Cancer Cell Growth

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To gain further insight into how antiestrogens modulate cell function, the effects of antiestrogen on cell proliferation were studied in human breast cancer cells. We examined the effects of trans-tamoxifen on the proliferation of three human breast cancer cell lines that differed in their estrogen receptor contents. Trans-tamoxifen (1 μM) markedly inhibited the estrogen stimulated proliferation of MCF-7 human breast cancer cells that contained high levels of estrogen receptor (1.15±0.03 pmole/mg protein) over that of control. In T47D cells that contained low levels of estrogen receptor (0.23±0.05 pmole/mg protein), trans-tamoxifen (1 μM) showed minimal inhibition of estrogen stimulated cell proliferation over that of control. MDA-MB-231 cells, that contained no detectable levels of estrogen receptors, had their growth unaffected by trans-tamoxifen treatment. These results showed their sensitivity to growth inhibition by antiestrogen correlated well with their estrogen receptor content. Also we examined the effect of antiestrogen on cellular progestrone receptor level as well as plasminogen activator activity in MCF-7 cells. Trans-tamoxifen (1 μM) showed maximal inhibition of estrogen stimulated progestrone receptor level as well as plasminogen activator activity in MCF-7 cells that were stimulated by estrogen. It is not clear whether these inhibitions of progestrone receptor and plasminogen activator activity by estrogen are related to the antiestrogen inhibition of cell proliferation of MCF-7 cells. From the results of this study, it is clearly demonstrated that trans-tamoxifen is an antiestrogen in MCF-7 human breast cancer cells. Our data suggest that the biological effectiveness of trans-tamoxifen appear to result from its affinity of interaction with the estrogen receptor.

Key words : trans-Tamoxifen, Estrogen receptor, MCF-7, T47D, MDA-MB-231, Progesterone receptor, Plasminogen activator activity

INTRODUCTION

Antiestrogens are compounds that antagonize the action of estrogens in a variety of estrogen target tissues. In particular, these compounds have been demonstrated to inhibit estrogen stimulated uterine growth in many animal species, to alter gonadotropin secretion, to cause the regression of hormone dependent mammary tumors and to inhibit the growth of estrogen receptor containing human breast cancer cells (Katzenellenbogen *et al.*, 1985). Although they were initially developed as fertility control agents for the human female, they are being employed successfully in the treatment of hormone dependent breast cancer due to their ability to control the growth of these cells. Antiestrogens, which generally have a triphenylethylene structure, are known to compete with estrogen for binding to estrogen receptor sites and the antiestrogen-occupied complex becomes localized in the cell nucleus (Horwitz and McGuire, 1978). The nuclear antiestrogen receptor complex, however, appears to be only partially active in promoting specific biological responses, and is effective in blocking the actions of estrogen (Katzellenbogen *et al.*, 1984). The determination of the estrogen and progesterone receptor content in human breast cancer tissue has gained an important role in the prediction of the success of an endocrine treatment (Nandi and McGrath, 1973). However, the receptor status of the tumor only allows a general evaluation; patients with a receptor-negative tumor respond rarely to an endocrine treatment; patients with receptor-positive tumors respond more frequently. An individual prediction in a single patient is not possible (Henderson, 1993; Bonadonna, 1993). Consequently, it is necessary to obtain a better understanding of the mechanism of action of trans-tamoxifen, which is the drug most often used in endocrine treatment of advanced breast cancer. It has been suggested that trans-tamoxifen exerts its effects on estro-

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gen-dependent breast cancer at least in part through the regulation of secreted polypeptide growth factors with growth stimulatory or inhibitory potential (Dickson *et al.*, 1993). Part of the effect of antiestrogens is thought to be mediated through the induction of transforming growth factor β (TGFβ), which has an autocrine growth inhibitory effect on most human breast cancer cells (Knabbe, 1991). While the precise mechanism by which antiestrogens evoke their antitumor effects is still incompletely understood, considerable experimental data are consistent with the hypothesis that antiestrogens exert their effects through the estrogen receptor system of the target cells (Katzell- enbogen *et al.*, 1985). In the MCF-7 human breast cancer cell line, which growth is estrogen dependent, estrogen stimulates cell proliferation, pS2 mRNA levels, plasminogen activator activity, thymidine incorporation, DNA synthesis, and progesterone receptor levels (Katzellenbogen *et al.*, 1985). Estrogen treatment of MCF-7 cells also results in the stimulation of two specific secreted proteins (Mr. 160,000 and 52,000) and a cytoplasmic protein (Mr. 24,000) (Westley and Rochefort, 1980; Veith *et al.*, 1983; Edwards, 1981). It has been postulated that the 52,000 Mr glycoprotein may be an estrogen-induced growth factor (Westley *et al.*, 1984), although data indicate that the 52,000 Mr protein may not be an autocrine regulator of MCF-7 cell growth (Davidson, 1986). To gain further insight into how antiestrogens modulate cell function, the effects of trans-tamoxifen on cell proliferation were studied in human breast cancer cells. We found that sensitivity to growth inhibition by antiestrogen correlates well with estrogen receptor contents.

MATERIALS AND METHODS

Chemicals and Materials

[^{3}H]Estradiol (106 Ci/mmol) and [^{3}H]trans-tamoxifen (60 Ci/mmol) were obtained from Amersham (Arlington Heights, IL, USA). The synthetic progestin [^{3}H]R5020 (17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione) (89 Ci/mmol) was obtained from New England Nuclear (Boston, MA, USA). All media, sera and antibiotics used to culture the MCF-7 cells were obtained from Grand Island Biological Co. (Grand Island, NY, USA). Insulin, hydrocortisone and trans-tamoxifen were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Bis (Cbz-Iso-Pro-Arg)-rhodamine was synthesized and purified according to the procedure of Leytus *et al.* (1984). Plasminogen was purified from fresh dog plasma by modification of the method of Castellino and Sodetz as described in Leytus *et al.* (1984). The toluene-based scintillation fluid was 0.5% 2,5-diphenyloxazole and 0.03% p-bis-[2-(5-phenyloxazoyl)]-benzene in toluene. The Triton-xylene-based scintillation fluid was 0.3% 2,5-diphenyloxazole, 0.02% p-bis[2-(5-phenyloxazoyl)]-benzene, and 25% Triton X-114 in xylene.

Cell Culture

MCF-7 human breast cancer cells were originally obtained from Dr. Charles McGrath of the Michigan Cancer Foundation (Detroit, MI, USA) and were grown in 60 mm plastic culture dishes in Eagles Minimal Essential medium (MEM) without phenol red supplemented with 10 mM HEPES buffer, gentamycin (50 μg/ml), penicillin (100 U/ml), streptomycin (1 mg/ml), bovine insulin (6 ng/ml), hydrocortisone (3.75 ng/ml), and 5% calf serum that had been treated with dextran-coated charcoal for 45 min at 55°C to remove endogenous hormones.

Estrogen Receptor Binding Analysis

Cells from 20 near-confluent 100 mm culture dishes were suspended in 2.0 mL of PTG buffer (5 mM sodium phosphate, pH 7.4 at 4°C, 10 mM thioglycerol, and 10% glycerol) and homogenized in a Dounce homogenizer using the B-pestle. The homogenate was centrifuged (800×g, 10 minutes) and the supernatant was collected. The crude nuclear pellet was washed twice at 0~4°C with buffer and the nuclear washes combined with the supernatant fraction. This was centrifuged at 180,000×g (30 minutes) to yield the cytosol which was diluted to 15.4 ml with PTG buffer. Aliquots of cytosol (200 μl) were incubated at 0~4°C for 20 h with [^{3}H]estradiol at concentrations ranging from 5×10^{-11} M to 5×10^{-9} M. Parallel tubes contained the radioactive ligand plus a 100-fold excess of radioinert estradiol to assess non-specific binding. An aliquot was withdrawn for determination of total radioactivity, and unbound ligand was then removed by incubating one part charcoal-dextran slurry (5% Norit A, 0.5% dextran in buffer) with nine parts extract for 8 minutes at 0~4°C. The charcoal was pelleted by a 3 minute centrifugation at 12,800×g, and an aliquot of the supernatant was withdrawn for counting.

Competitive Binding Assays with Estrogen Receptor

Cells were harvested from 10 cm culture dishes and cytosol was prepared at a protein concentration of ca. 1.5 mg/ml in PTG buffer. An aliquot of this 180,000×g supernatant was then incubated with various concentrations of radioinert estradiol or trans-tamoxifen and 5×10^{-9} M [^{3}H]estradiol at 0~4°C for 16 h and samples were then analyzed.

Cell Proliferation Experiments

The effect of etradiol on cell proliferation was studied in MCF-7, T47D and MDA-MB-231 cells. MCF-

7, T47D and MDA-MB-231 cells were seeded into 35 mm culture dishes (20×10^3 cells/dish) and grown for two days in the MEM medium described in the Cell Culture section above. After this time, cells from two flasks were harvested and counted with a Coulter Counter (Day 0). The medium was changed to MEM supplemented as described above except containing 2% charcoal-dextran treated calf serum and various concentrations of trans-tamoxifen or ethanol vehicle (0.1%). Triplicate dishes of cells were counted at several points throughout the 13 day growth period.

Hydroxylapatite Assay of Progesterone Receptor

Cytosol was incubated at 0~4°C for 4 h with 10 nM [^{3}H]R5020 in the presence or absence of 1 μM radioinert R5020, a synthetic progestin. Pretreatment of cytosol with 10^{-6} M cortisol, prior to incubation with [^{3}H]R5020 had no effect on the level of progestin binding, suggesting no contribution from glucocorticoid receptor. After incubation, samples were assayed for bound [^{3}H]R5020 using hydroxylapatite.

Assay for Plasminogen Activator Activity

The plasminogen activator activity of the cells was measured by a two-step assay using the rhodamine-based compound Bis (Cbz-Ile-Pro-Arg)-rhodamine, abbreviated BZIPAR, as a substrate for plasmin. The substrate is non-fluorescent but cleavage by plasmin of an amide bond between arginine and rhodamine yields a product mono (Cbz-Ile-Pro-Arg)-rhodamine that is highly fluorescent. In brief, cells were incubated with plasminogen for 2 h at 37°C and the amount of plasmin formed was then measured by incubating an aliquot of the reaction mixture with BZIPAR and determining the rate of increase in fluorescence. The rate of activation of plasminogen during the 2 h incubation of plasminogen with cells was found to be constant and, therefore, proportional to the amount of plasminogen activator. Conversion of relative fluorescence units per minute to molar concentrations of plasmin was accomplished by using a standard curve.

RESULTS AND DISCUSSION

Effects of trans-tamoxifen on proliferation of breast cancer cells *in vitro*

We examined the effects of trans-tamoxifen on the proliferation of three human breast cancer cell lines that differed in their estrogen receptor contents. As shown in Fig. 1, trans-tamoxifen (1 μM) markedly inhibited the estrogen stimulated proliferation of MCF-7 human breast cancer cell that contained high levels of estrogen receptor over that of control when trans-tamoxifen was administered into cell concomitantly with inhibited estrogen. However, trans-tamoxifen alone treatment did not change the cell numbers compared to that of control. In T47D cells that contained low levels of estrogen receptor, trans-tamoxifen (1 μM) showed minimal inhibitory effect on the estrogen stimulated cell proliferation over that of control (Table I, Fig. 2). MDA-MB-231 cells, that contained no detectable levels of estrogen receptors, had their growth unaffected by trans-tamoxifen (Fig. 3). These results

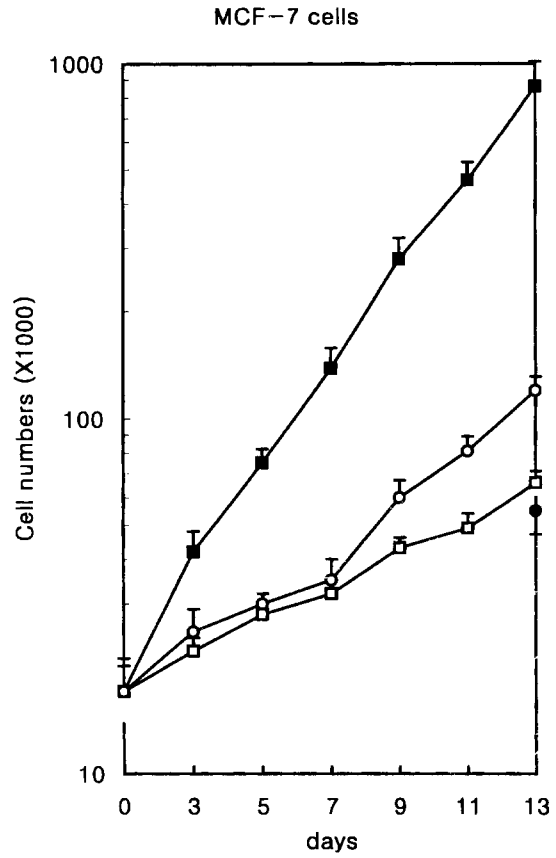

Fig. 1. Effect of trans-tamoxifen on the growth of MCF-7 cells. Cells were grown in the continuous presence of 1 μM trans-tamoxifen in the presence or absenece of 10 nM estradiol, and media with fresh trans-tamoxifen were renewed every other day. On the days indicated, triplicate dishes of cells were counted. Values are the means of the triplicate determinations. Bars represent S.E. ○: estradiol+trans-tamoxifen, ●: trans-tamoxifen, □: estradiol.

Table I. Estrogen receptor levels in human breast cancer cell lines. Measurement of trans-tamoxifen receptor was carried out as described in materials and methods

cells	estrogen receptor (pmole/1 mg protein)
MCF-7	1.15±0.03
T47D	0.23±0.05
MDA-MB-231	not detected

mean±S.E. n=6

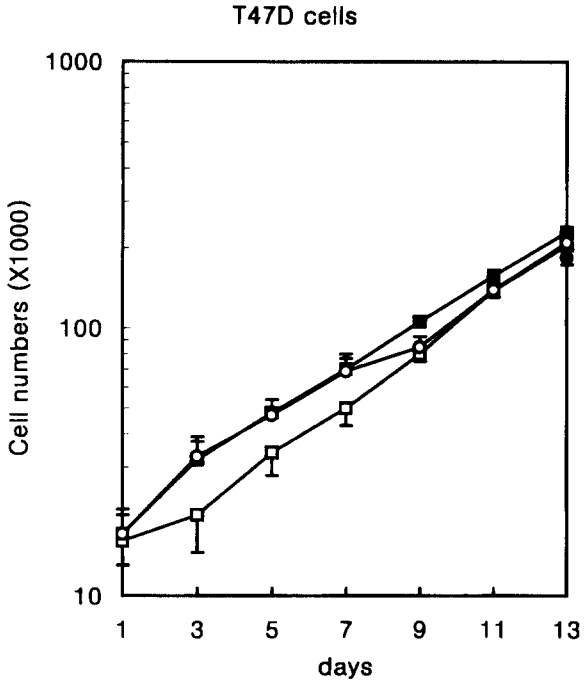

Fig. 2. Effect of trans-tamoxifen on the growth of T47D cells. Cells were grown in the continuous presence of 1 μM trans-tamoxifen in the presence or absenece of 10 nM estradiol, and media with fresh trans-tamoxifen were renewed every other day. On the days indicated, triplicate dishes of cells were counted. Values are the means of the triplicate determinations. Bars represent S.E. ○: estradiol+trans-tamoxifen, ●: trans-tamoxifen, □: estradiol.

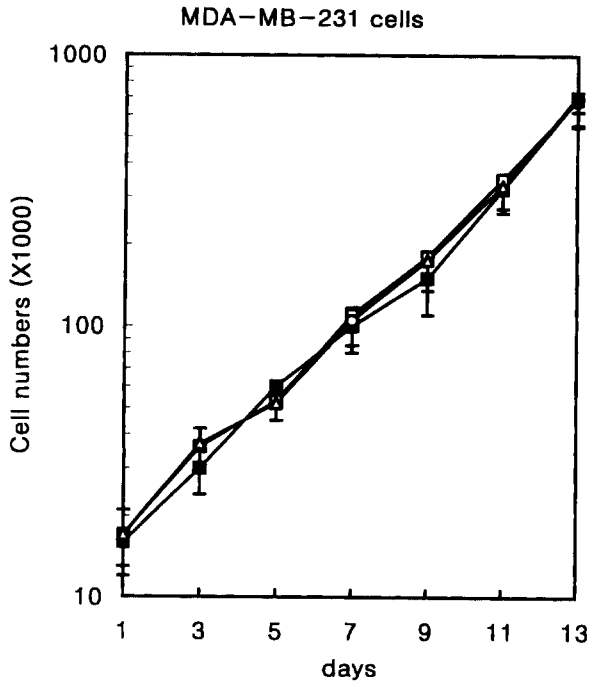

Fig. 3. Effect of trans-tamoxifen on the growth of MDA-MB-231 cells. Cells were grown in the continuous presence of 1 μM trans-tamoxifen in the presence or absenece of 10 nM estradiol, and media with fresh trans-tamoxifen were renewed every other day. On the days indicated, triplicate dishes of cells were counted. Values are the means of the triplicate determinations. Bars represent S.E. ○: estradiol+trans-tamoxifen, ●: trans-tamoxifen, □: estradiol.

showed their sensitivity to growth inhibition by antiestrogen correlated well with their estrogen receptor contents. These findings are mirrored by the results with human breast cancer patients indicating that estrogen receptor-containing breast cancers are most sensitive to antiestrogen treatment (McGuire, 1979). Studies with antiestrogen in human breast cancer cells in culture indicate that antiestrogen selectively inhibits the proliferation of estrogen receptor-containing breast cancer cells.

Analysis of the binding of trans-tamoxifen to MCF-7 estrogen receptor

Based on data of saturation binding analysis for estradiol in MCF-7 cells (Lee and Sheen, submitted to *Arch. Pharm. Res*, 1997) the presence of a single class of high affinity binding site in MCF-7 cell cytosol with equilibrium dissociation constants (Kd) of 0.25 nM for estradiol was observed. Estrogen receptor levels in human breast cancer cell lines were compared (Table I). As shown in Table I, MCF-7 cell contains high level of estrogen receptor and T47D cell contains low level of estrogen receptor. However, MDA-MB-231 cell contains no detectable estrogen receptors. The relative binding affinity of trans-tamoxifen for the estrogen receptor was also determined indirectly by competitive binding analyses (Fig. 4A, 4B). Comparison of the concentrations of trans-tamoxifen and estradiol needed to decrease the specific binding of tritiated estradiol by 50% indicates that trans-tamoxifen has an affinity of 6% compared to that of estradiol. In addition, the full displacement of [^{3}H]estradiol binding by unlabeled trans-tamoxifen (Fig. 4A, 4B) and the full displacement of [^{3}H]trans-tamoxifen binding by unlabeled estradiol (Fig. 4A, 4B) indicate that the trans-tamoxifen and estradiol bind in a mutually competitive manner to the MCF-7 estrogen receptor.

Effect of trans-tamoxifen on cellular progesterone receptor content

Effect of trans-tamoxifen on cellular progesterone receptor content was examined in MCF-7 cells. Increase in progesterone receptor content had been used as an index of estrogen action in MCF-7 cells (Eckert and Katzellenbogen, 1982). Our previous study showed that maximal effect of estrogen on the levels of progesterone receptor was observed at 5-day time point (Lee and Sheen, 1997). Hence, 5-day time point was selected for evaluation of antiestrogen inhibition on estrogen stimulated progesterone receptor. Fig. 5 shows

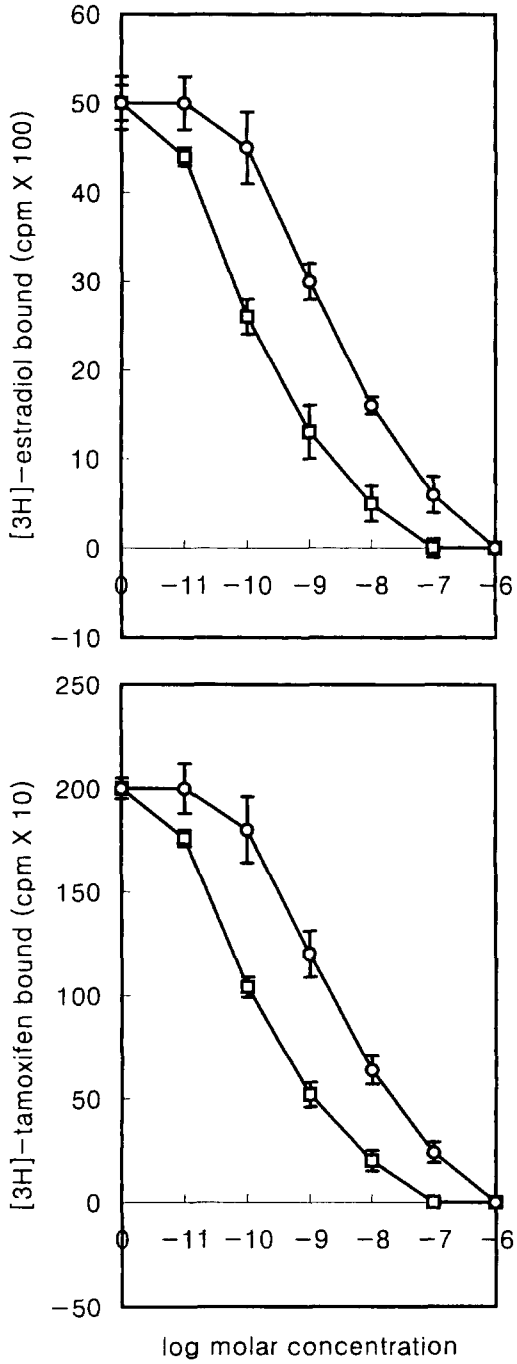

Fig. 4. Analysis of the binding of [^{3}H] estradiol to MCF-7 estrogen receptor. Cytosol was incubated with varying concentrations of nonradiolabeled estradiol or trans-tamoxifen from 5×10^{-11} M to 5×10^{-9} M, in the presence of [^{3}H] estradiol 5×10^{-9} (A) or [^{3}H] trans-tamoxifen 5×10^{-8} M (B) for 16 hours at 0~4°C. An aliquot was removed to assess total binding, and bound radioactivity was determined following charcoal-dextran treatment. Protein concentration in the cytosol incubation was 1.0 mg/ml. ○: trans-tamoxifen, □: estradiol.

a dose dependent decrease in estrogen stimulated progesterone receptor by trans-tamoxifen treatment when 1 μM trans-tamoxifen was treated with 10 nM estradiol

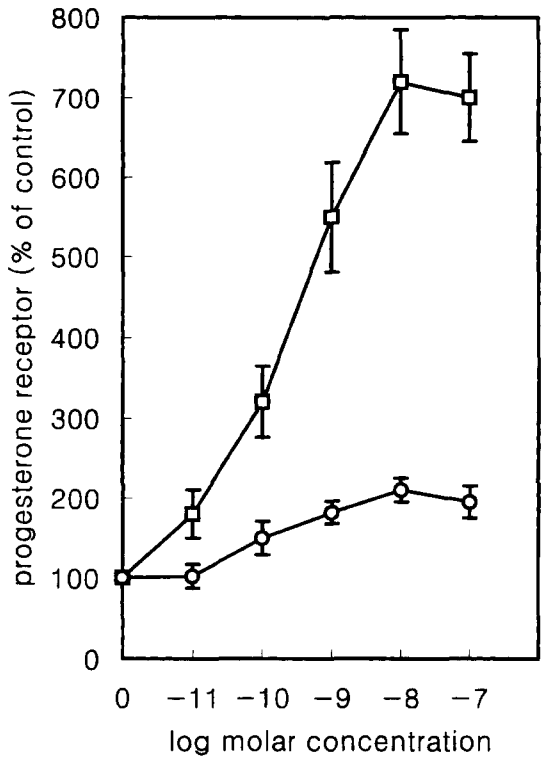

Fig. 5. Effect of trans-tamoxifen on progesterone receptor levels in MCF-7 cells. Cells were incubated for 5 days with trans-tamoxifen in the presence or absenece of 10nM estradiol at the concentrations indicated. Fresh media and trans-tamoxifen were added daily during 5-days period. The cells were then harvested, fractionated, and the cytosol was assayed for progesterone receptor using hydroxylapatite, as described in "Materials and Methods". Control progesterone receptor level was 330±32 fmol/mg DNA. ○: estradiol+trans-tamoxifen, ●: trans-tamoxifen, □: estradiol.

concomitantly, whereas estrogen treatment stimulated, 6-fold increase in progesterone receptor level. This data confirms that antiestrogen inhibits progesterone receptor synthesis by estrogen.

Effect of trans-tamoxifen on plasminogen activator activity

Trans-tamoxifen inhibits estrogen stimulated plasminogen activator activity with dose dependent manner when trans-tamoxifen was treated with 10 nM estradiol, whereas trans- tamoxifen alone treatment resulted no changes in plasminogen activator activity level (Fig. 6). This data also confirms that antiestrogen inhibits estrogen stimulated plasminogen activator activity. It has been demonstrated previously that plasminogen activator activity serves as a useful marker for the biological activity of estrogen in human breast cancer cells (Butler *et al.*, 1983; Kneifel *et al.*, 1982). Plasminogen activator activity is stimulated by low, physiological concentrations of estradiol in MCF-7 cells, while antiestrogen such as tamoxifen and trans-

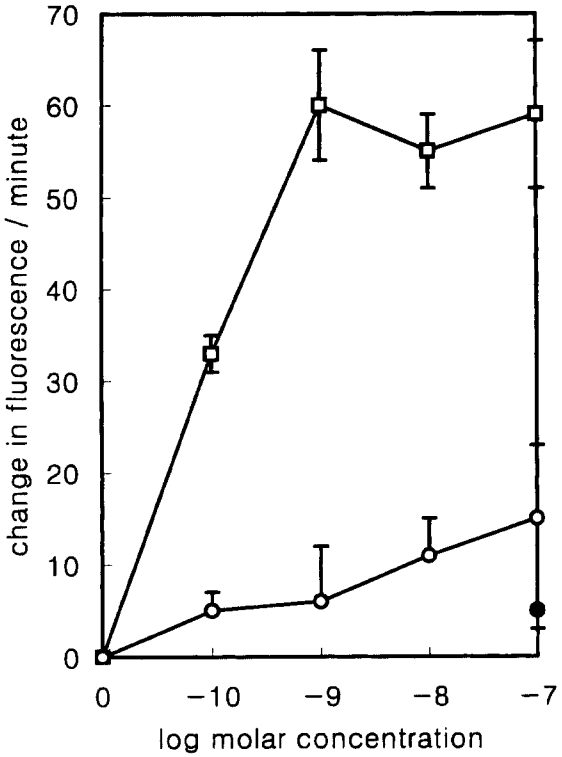

Fig. 6. Effect of trans-tamoxifen on the plasminogen activator activity of MCF-7 cells. Cells were incubated with the indicated concentration of trans-tamoxifen in the presence or absenece of 10 nM estradiol for 4 days, with fresh media and trans-tamoxifen renewed every 48 hours. Cells were then harvested, distributed to microwells, and assayed for plasminogen activator activity, was measured by the change in fluorescence (△F) per minute, representing plasmin formed per minute; bars, S.E. ○: estradiol+trans-tamoxifen, ●: trans-tamoxifen, □: estradiol.

hydroxytamoxifen failed to stimulate plasminogen activator activity (Butler *et al.*, 1983). Plasminogen activator activity is under hormonal control in many tissues, and increased activity is found during rapid tissue growth and remodeling of the uterus and mammary gland (Kneifel *et al.*, 1982). The results of these studies indicate trans-tamoxifen suppressed estrogen stimulation of MCF-7 cell proliferation, plasminogen activator activity and progesterone receptor. The potency in growth suppression and plasminogen activator activity by trans-tamoxifen appeared to correlate well with the affinity of trans-tamoxifen for the estrogen receptor. Furthermore, as shown in Fig. 1, growth suppression by trans-tamoxifen could be reversed by estradiol, suggesting mediation by an estrogen-competetitive process, presumably the estrogen receptor. Recent studies have revealed that triphenylethylene compounds, such as tamoxifen, bind with high affinity to both the estrogen receptor and to additional sites to which estrogen do not bind. These latter sites have been termed "antiestrogen-specific binding sites". These sites have a subcellular distribution and binding specificity that is quite different from that of the estrogen receptor. In addition, these antiestrogen-specific binding sites are found in an equal quantity in three human breast cancer cell lines (MCF-7, T47D, and MDA-MB-231) that differ significantly in their sensitivity to growth inhibition by antiestrogen (Sudo *et al.*, 1983). The role of this additional antiestrogen binding component for which the Kd for tamoxifen is approximately 3 nM (Lee and Sheen, 1997) is not known at present. From the results of this study, it is clearly demonstrated that trans-tamoxifen is an antiestrogen in MCF-7 human breast cancer cells. Our data suggest that the biological effectiveness of trans-tamoxifen appear to result from its affinity of interaction with the estrogen receptor.

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REFERENCES CITED

Bonadonna, G. What did we learn from the results of the international overview about the effects of endocrine therapy? *Recent Results Cancer Res.*, 127, 159-161 (1993).

Butler, W. B., Kirkland, W. L., Gayala, T. L., Goran, N., Kelesey, W. H. and Berkinski, P. J. Steroid stimulation of plasminogen activator production in a human breast cancer cell line (MCF-7). *Cancer Res.*, 43, 1637-1644 (1983).

Davidson, N. E., Bronzert, D. A., Chambon, P., Gelmann, E. P. and Lippman, M. E. Use of two MCF-7 cell variants to evaluate the growth regulatory potential of trans-tamoxifen-induced products. *Cancer Res.*, 46, 1904-1909 (1986).

Dickson, R. B., Thompson, E. W. and Lippman, M. E. Regulation of proliferation, invasion, and growth factor synthesis in breast cancer by steroids. *J. Steroid Biochem. Mol. Biol.*, 37, 305-316 (1990).

Eckert, R. L. and Katzellenbogen, B. S. Effects of trans-tamoxifens and antitrans-tamoxifens on trans-tamoxifen receptor dynamics and the induction of progesterone receptor in MCF-7 human breast cancer cells. *Cancer Res.*, 42, 139-144 (1982).

Edward, D. P., Adams, D. J. and McGuire, W. L. Estradiol stimulates synthesis of a major intracellular protein in the human breast cancer cell line MCF-7. *Breast Cancer Res. Treat.*, 1, 209-229 (1981).

Henderson, I. C. Adjuvant systemictherapy; overview. *Recent Results Cancer Res.*, 127, 197-200 (1993).

Horwitz, K. B. and McGuire, W. L. Nuclear mechanism of estrogen receptor action. *J. Biol. Chem.*, 253, 8185-8191, (1978).

Katzellenbogen, B. S., Miller, M. A., Mullick, A. and Sheen, Y. Y. Antitrans-tamoxifen action in breast cancer cells. *Breast Cancer Res. and Treatment,* 5, 231-243 (1985).

Katzellenbogen, B. S., Norman, M. J., Eckert, R. L., Peltz, S. W. and Mangel, W. F. Bioactivity eestrogen receptor interactions and plasmin activator inducing activities of tamoxifen and hydroxytamoxifen isomers in MCF-7 breast cancer cells. *Cancer Res.,* 44, 112-119 (1984).

Katzellenbogen, B. S. Uterine plasminogen activator: modulation by steroid hormones. *Endocrinology,* 111, 493-500 (1982).

King W. J. and Green G. L. Monoclonal antibodies localize trans-tamoxifen receptor in nuclei of target cells. *Nature,* 307, 745-747 (1984).

Knabbe, C. Regulation of growth inhibitory polypeptides in human breast cancer. In Voigt, K. D. and Knabbe, C. (Eds.). *Endocrine-dependent Tumors.* Raven Press, New York. pp 165-178, 1991.

Kneifel, M. A., Leytus, S. P., Fletcher, E., Weger, T., Mangel, W. F. and Leytus, S. P., Toledo, D. L. and Mangel, W. F. Theory and experimental method for determining kinetic constants of fast-acting irreversible protease inhibitors. *Biochem. Biophys. Acta,* 788, 74-86 (1984).

Lee, H. O. and Sheen, Y. Y. Estrogen modulation of breast cancer cells. *Arch. Pharm. Res.,* in press (1997).

McGuire, W. L. Steroid receptor sites in cancer therapy. *Adv. Intern. Med.,* 24, 127-140 (1979).

Nandi, S. and McGrath, C. M. Mammary neoplasia in mice. *Adv. Cancer Res.,* 17, 354-414 (1973).

Schatchard, G. The attractions of protein for small molecule and ions. *Ann. NY Acad. Sci.,* 51, 660-672 (1949).

Sudo, K., Mondma, F. J. and Katzellenbogen, B.S. Antiestrogen binding sites are distinct from estrogen receptor. *Endocrinology,* 112, 425-434 (1983).

Veith, F. O., Capony, F., Garcia, M., Chantelard, J., Pujol, H., Veith, F., Zajdela and Rochefort, H. Release of trans-tamoxifen-induced glycoprotein with a molecular weight of 52,000 by breast cancer cells in primary culture. *Cancer Res.,* 43, 1861- 1867 (1983).

Welshons W. V., Liebeman, M. E. and Gorski, J. Nuclear localization of unoccupied trans-tamoxifen receptors. *Nature,* 307, 747-749 (1984).

Westley, B., May, F. E. B., Brown, M. C., Krust, A., Chambon, P. and Rochefort, H. Effect of antitrans-tamoxifens on the trans-tamoxifen regulated pS2 RNA and the 52- and 160-kilodalton proteins in MCF-7 cells and two tamoxifen-resistant sublines. *J. Biol. Chem., 259,* 10030-10036 (1984).

Westley, B. and Rochefort, H. A secreted glycoprotein induced by trans-tamoxifen in human breast cancer cell lines. *Cell,* 20, 353-358 (1980).