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ORIGINAL ARTICLE

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Antiestrogenic activity of DP-TAT-59, an active metabolite of TAT-59 against human breast cancer

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Abstract *Purpose*: The purpose of this study was to clarify the mechanism(s) of antiestrogenic action of DP-TAT-59 ((*Z*)-2-(4-(1-(4-hydroxyphenyl)-2-(4-isopropyl-phenyl)-1-butenyl)phenoxy)-*N*,*N*-dimethylethylamine), the main active metabolite of TAT-59. *Methods*: Using 4-OH-tamoxifen (a hydroxylated metabolite of tamoxifen) as a reference compound, we examined the relationship between hormone-dependent tumor cells and DP-TAT-59 and characterized estrogen receptor (ER) complexes with DP-TAT-59 using ion-exchange chromatography. *Results*: DP-TAT-59 inhibited the in vitro proliferation of MCF-7 cells under serum-free conditions at a lower concentration than did 4-OH-tamoxifen. The conditioned medium (CM) obtained from the culture supernatant of MCF-7 cells in the presence of these antiestrogens suppressed the growth of ER-negative cell lines, but that from ER-negative human mammary carcinoma MX-1 cells did not. The CM from DP-TAT-59-treated cells showed a higher growth-inhibitory potency against human mammary carcinoma ZR-75-1 cells than did that from 4-OH-tamoxifen-treated cells. The growth-inhibitory potency of the CM was neutralized by the addition of the anti-TGF-β antibody. The CM obtained from cells treated with DP-TAT-59 contained more TGF-β and less TGF-α than that treated with 4-OH-tamoxifen. As the antiestrogenic activity of TAT-59 might be mediated through ER, the interaction of these antiestrogens with a cytoplasmic receptor of MCF-7 cells was examined. While the competitive binding of [^{3}H]-estradiol with these antiestrogens to ER was similar, ER complexes with DP-TAT-59 showed a different elution profile by ion-exchange chromatography, indicating that DP-TAT-59 formed a different complex with ER from either 4-OH-tamoxifen or estradiol. *Conclusion*: These findings suggest that at least a part of the growth suppressive ability of DP-TAT-59 against human mammary carcinoma might depend on the production of growth inhibitory factors and/or the suppression of production of growth factors from ER-positive cells, and that the production of growth inhibitory factors might be stimulated by ER complexes with antiestrogens rather than with estrogen.

Key words TAT-59 · Tamoxifen · Antiestrogenic activity · ER complex · Growth inhibitory factor

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Introduction

The involvement of hormones in some human breast cancers has been known for many years and endocrine therapy has been recognized as a useful treatment for this cancer. In particular, triphenylethylene derivatives such as tamoxifen have been found to be an effective additive therapy in the treatment of hormone-dependent breast cancer [15]. We have synthesized several new triphenylethylene derivatives, which have been screened with the use of a hormone-dependent tumor model leading to the selection of TAT-59 [2]. As a result of the superior antitumor activity of TAT-59 over tamoxifen against in vivo experimental tumors even those with a low estrogen receptor (ER) content [3], clinical studies on TAT-59 have recently been initiated.

The mechanism of action of antiestrogens is still unclear. The formation of ER complexes with antiestrogens is thought to be the initial and essential step of antiestrogenic action. The growth inhibitory effect of antiestrogens is reportedly related to their affinity for ER [3, 21, 26]. A different conformation of ER complexes with antiestrogens from that with estradiol has

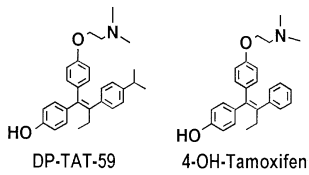


Fig. 1 Chemical structure of DP-TAT-59 and 4-OH-tamoxifen

been suggested as contributing to antiestrogenic activity [12, 22, 25, 28]. Estradiol stimulates the growth of hormone-dependent cells by the production of growth factors such as transforming growth factor α (TGF-α) [7], insulin-like growth factor II (IGF-II) [30], and platelet-derived growth factor (PDGF) [5]. In contrast, once estrogen-dependent cells are exposed to an antiestrogen, either a reduction in the production of growth factor(s) [8] or an increase in the production of growth inhibitory factor(s) [14] occurs.

To clarify the mechanism(s) of the antiestrogenic action of DP-TAT-59 (Fig. 1), the main active metabolite of TAT-59 [29], we examined the relationship between hormone-dependent tumor cells and DP-TAT-59 and characterized the ER complexes with DP-TAT-59, using 4-OH-tamoxifen (Fig. 1) as a reference compound.

Materials and methods

Materials

[2,4,6,7-^{3}H]-Estradiol (115 Ci/mmol) and (*Z*)-[*N*-methyl-^{3}H]-4-OH-tamoxifen (85 Ci/mmol) were obtained from Amersham International (Amersham, UK). [^{3}H]-DP-TAT-59 (43.2 Ci/mmol) was synthesized by Amersham International. Tamoxifen and 17β-estradiol were purchased from Sigma Chemicals (St. Louis, Mo.). 4-OH-Tamoxifen and DP-TAT-59 were prepared in our laboratory. Estrogen and antiestrogens were first dissolved in dimethylsulfoxide (DMSO) to prepare stock solutions which were diluted with buffer or culture medium. Growth factors (TGF-α, TGF-β) and antibodies (anti-TGF-α, anti-TGF-β) were obtained from R&D Systems (Minneapolis, Minn.). ABC kits for measurement of growth factors were purchased from Vector Laboratories (Burlingame, Calif.). DEAE-Sephadex A-25 (Pharmacia LKB, Uppsala, Sweden) was used for ion-exchange column chromatography. MCF-7 cells were maintained in RPMI-1640 medium (Flow Laboratories, Irvine, UK) supplemented with 10% fetal calf serum (FCS), 1 μg/ml insulin (Sigma Chemicals) and 10^{-9} *M* estradiol, and were used for experiments in Ham's F-12 medium comprising Dullbecco's modified MEM (1:1; Flow Laboratories) containing 0.1% bovine serum albumin (BSA; Pharmacia LKB), 5 ng/ml epidermal growth factor (EGF; Earth Pharmaceutical, Tokyo, Japan) and 10^{-9} *M* estradiol.

Inhibition of the growth of MCF-7 cell

The culture medium was replaced 7 days before starting the experiments with estradiol-depleted serum-free medium. Cells were detached with 0.25% trypsin and 1 m*M* EDTA, pelleted, resuspended in medium without estradiol and plated in a 24-well multiplate (Becton Dickinson, Bedford, Mass.) at a concentration of 3000 cells/well. After cell attachment (20–24 h), drugs were added to the medium. The final concentration of DMSO (0.1%) had no effect on cell growth. After a further 10 days incubation, the cells were fixed with glutaraldehyde and stained with crystal violet. The percentage change in cell count was evaluated based on the concentration of dye extracted from the stained cells.

Whole-cell binding assay

MCF-7 cells (2.5×10^5 cells/well) were attached into a 24-well multiplate. To produce an equilibrium between intra- and extracellular concentrations of antiestrogens, the medium was replaced with RPMI-1640 medium containing 0.1% BSA and increasing concentrations of unlabeled estradiol or antiestrogens. After replacement of the medium, the cells were exposed to 5 n*M* [^{3}H]-estradiol at 37 °C. The medium was then removed and the cell monolayer was washed twice with ice-cold phosphate buffered saline (PBS) containing 0.5% BSA and 10% glycerol (PBS-A). Radioactivity was extracted with ethanol and measured using a liquid scintillation analyzer (Tri-Carb 2000CA, Packard Instrument Meriden, Conn. Co.).

The binding of estradiol to the cytoplasmic receptor of intact MCF-7 cells was characterized by Scatchard analysis. The Kd value was 6.4×10^{-10} *M* and the number of binding sites was 8.24 fmol/μg DNA.

Cellular uptake of antiestrogens

MCF-7 cells (2.5×10^5 cells/well) were plated into a 24-well multiplate and allowed to attach for 48 h and exposed to 5 n*M* [^{3}H]-estradiol or [^{3}H]-antiestrogens. After various incubation periods, cells were washed three-times with ice-cold PBS-A. The incorporated radioactivity was extracted with ethanol and measured using a liquid scintillation analyzer.

DEAE-Sephadex column chromatography

MCF-7 cells were homogenized in 3 volumes of TE buffer (10 m*M* Tris, 1.5 m*M* EDTA, 10 m*M* monothioglycerol, 0.5 m*M* PMSF, 10 m*M* molybdate, pH 7.5) using a Biotron homogenizer. The homogenate was then centrifuged at 100 000*g* for 1 h to obtain a cytosol. The cytosol was incubated on ice for 2 h with either [^{3}H]-DP-TAT-59, [^{3}H]-4-OH-tamoxifen or [^{3}H]-estradiol both in the absence and the presence of a 200-fold molar excess of estradiol. The labeled cytosol was mixed with a pellet from an equal volume of dextran-coated charcoal (DCC, 0.5% activated charcoal and 0.05% dextran in TE buffer) for 15 min and centrifuged at 3000 rpm for 25 min at 4 °C. DEAE-Sephadex A-25 5-ml columns were washed with 50 volumes of TE buffer. The labeled cytosols were loaded onto the columns and washed with 25 ml TE buffer. Elution was performed using 90 ml of a 0–0.4 *M* linear gradient of KCl in TE buffer at 1 ml/min. The eluent was collected in 2-ml fractions and the radioactivity was measured.

Growth inhibitory activity in conditioned medium from MCF-7 cells

Subconfluent monolayers of MCF-7 cells or MX-1 cells grown in serum-free medium were treated for 2 days with either DP-TAT-59 or 4-OH-tamoxifen at a range of concentrations from 10^{-8} to 10^{-13} *M* in 25-cm^2 culture flasks (Becton Dickinson). Serum-free conditioned medium (CM) was concentrated tenfold by ultrafiltration with Centriflo membrane cones (type CF-25, Amicon). The CM was applied to monolayer cultures of the indicator cell lines

MX-1 (5000 cells/well), ZR-75-1 (2500 cells/well) and Mv-1-Lu (2000 cells/well) in a 96-well multiplate. After a 4-day exposure, the growth inhibitory activity of the CM was evaluated based on the number of indicator cells.

Growth-inhibition of indicator cells by coculture of MCF-7 cells treated with antiestrogen

MX-1 (5000 cells/well), ZR-75-1 (3000 cells/well) and Mv-1-Lu cells (3000 cells/well) were seeded into a 24-well multiplate as indicator cells and MCF-7 cells (8×10^4 cells) into culture inserts for the 24-well multiplate with a 0.45-μm transparent membrane (Becton Dickinson). One day after seeding, various concentrations of compounds were added to the medium, and 7–10 days after treatment, the growth of the indicator cells was estimated by the protein staining method using crystal violet, as described above.

The proliferation rate of the indicator cells was not affected by the antiestrogens at the concentrations used in these experiments.

Growth factor contents in the conditioned medium

Subconfluent monolayers of MCF-7 cells were treated with DP-TAT-59, tamoxifen and 4-OH-tamoxifen at a range of concentrations from 10^{-7} *M* to 10^{-12} *M* for 24 h. ELISA was used to determine the concentration of growth factors in 100 μl of the CM. For measurement of TGF-β, the CM was treated with 0.03 *N* HCl before analysis in the ELISA.

Neutralization of growth inhibitory activity in conditioned medium by anti-TGF-β antibody

CM from MCF-7 cells treated with 10^{-7} to 10^{-12} *M* antiestrogens was applied to Mv-1-Lu cells (3000 cells/well) in the presence or absence of 1 μg/ml anti-TGF-β antibody. After a 6-day exposure, the growth-inhibitory activity of the CM was evaluated based on the number of Mv-1-Lu cells as determined by the protein staining method.

Results

Inhibition of the growth of MCF-7 cells

Figure 2 shows the effects of antiestrogens on the growth of MCF-7 cells. DP-TAT-59 and 4-OH-tamoxifen inhibited the estrogen-stimulated growth of MCF-7 cells in a dose-dependent manner at a range of concentrations from 10^{-6} to 5×10^{-11} *M*. The growth-inhibitory activity of DP-TAT-59 was seen at lower concentrations than that of 4-OH-tamoxifen or tamoxifen. About 10- and 100-fold higher concentrations of 4-OH-tamoxifen and tamoxifen were necessary to achieve the same effect, respectively.

Competitive binding of antiestrogens by whole-cell binding assay

The different sensitivity of MCF-7 cells to antiestrogens prompted us to characterize the competitive binding ability of DP-TAT-59, 4-OH-tamoxifen and estradiol against 5 n*M* [^{3}H]-estradiol to intact MCF-7 cells, in the presence of varying concentrations of nonradioactive ligands. As shown in Fig. 3, the binding of [^{3}H]-estradiol to receptor was inhibited by DP-TAT-59, 4-OH-tamoxifen and estradiol, whereas the concentration of these antiestrogens necessary to achieve 50% inhibition of the binding of [^{3}H]-estradiol to receptor was about tenfold higher than that of estradiol. There was no significant difference in inhibition of binding of [^{3}H]-estradiol to receptor between the antiestrogens.

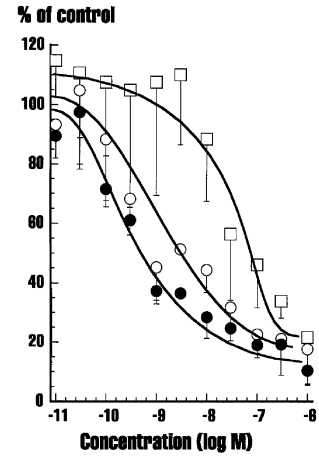


Fig. 2 Inhibitory effect of antiestrogens on the growth of MCF-7 cells under serum-free conditions. The values shown are the means and SD from two independent experiments (● DP-TAT-59, ○ 4-OH-tamoxifen, □ tamoxifen)

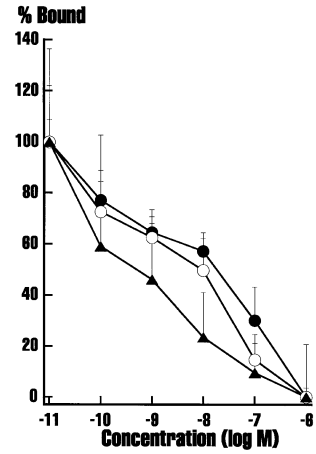


Fig. 3 Competitive binding of 5×10^{-9} *M* [^{3}H]-estradiol in the presence of different concentrations of estrogen and antiestrogens. The values shown are the means and SD from more than three determinations (● DP-TAT-59, ○ 4-OH-tamoxifen, ▲ estradiol)

Cellular uptake of antiestrogens

Triphenylethylene derivates have been reported to have binding sites different from that of estrogen [4, 24]. Therefore, we examined the cellular uptake of antiestrogens and equilibrated the concentration of antiestrogens between the intra- and extracellular spaces during the period of the whole-cell binding assay. As shown in Fig. 4, a steady-state was achieved

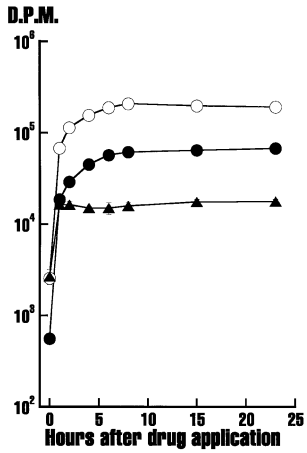


Fig. 4 Cellular uptake of estrogen and antiestrogens by MCF-7 cells. The values shown are the means and SD of four determinations (● DP-TAT-59, ○ 4-OH-tamoxifen, ▲ estradiol)

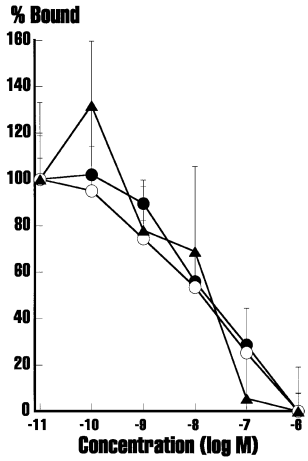


Fig. 5 Competitive binding of 5×10^{-9} *M* [^{3}H]-estradiol after preincubation of MCF-7 cells with different concentrations of estrogen and antiestrogens. The values shown are the means and SD of three determinations (● DP-TAT-59, ○ 4-OH-tamoxifen, ▲ estradiol)

after 8 h of incubation but not after a shorter incubation period, for example 1 h. At steady-state, the amount of incorporated antiestrogens was about four-fold higher than that of estradiol. We assume that antiestrogens possess about four times more receptors than estradiol, providing that other factors such as the efflux mechanism and physicochemical properties are similar to those of estradiol.

Whole-cell binding assay under long-term incubation

We re-examined the whole-cell assay using an 8-h incubation to reach an equilibrium state. In contrast to the result obtained from short-term incubation, the inhibitory activity of antiestrogens against the binding of [^{3}H]-estradiol was similar to that of estradiol (Fig. 5). We conclude that these three compounds bound to cytoplasmic ER with similar affinity under steady-state conditions.

Analysis of receptor complexes by DEAE-Sephadex column chromatography

Although there was no difference in the ligand-receptor binding capacity among antiestrogens, because the characteristics of the interaction between ligand and receptor were defined not only by their binding capacity but also by the combination of the complexes formed, the differences between the receptor complexes formed were detected by ion-exchange chromatography using DEAE-Sephadex columns. As shown in Fig. 6, column chromatography of the receptor complexes labeled with [^{3}H]-estradiol yielded only one peak at 0.18 *M* KCl (peak A) which was found in elution profiles of both [^{3}H]-DP-TAT-59 and [^{3}H]-4-OH-tamoxifen at a similar concentration of KCl. However, an additional peak was eluted at approximately 0.10 *M* KCl from receptor complexes labeled with antiestrogens (peak B). Comparison of the ratio of peak areas with 0.10 *M* KCl and with 0.18 *M* KCl, showed clear quantitative differences between ER complexes of DP-TAT-59 and 4-OH-tamoxifen. When MCF-7 cells were treated with each tritiated ligand in the presence of a 200-fold excess of cold estradiol, all peaks disappeared owing to displacement of the radiolabeled ligands from their cytoplasmic receptor complex. These findings suggest that both DP-TAT-59 and 4-OH-tamoxifen formed different receptor complexes from the receptor complex formed with estradiol and that DP-TAT-59 formed these different receptor complexes at a higher ratio than did 4-OH-tamoxifen.

Growth inhibitory activity in medium from MCF-7 cells treated with antiestrogen

Since the growth inhibitory activity of antiestrogens has been reported to be mediated through the production of growth inhibitor(s) [14], which might be a result of the formation of different ER complexes with antiestrogen from that with estrogen, we examined the growth inhibitory activity against human mammary carcinoma in culture medium derived from MCF-7 cells treated with antiestrogens. The CM obtained from ER-positive MCF-7 cells treated with antiestrogens suppressed the growth of Mv-1-Lu cells (Fig. 7A) but neither the CM from MCF-7 cells treated with 10^{-9} *M* of DP-TAT-59 in the presence of 10^{-9} *M* of estradiol (column DP + E2 in Fig. 7A) nor the CM from ER-negative MX-1 cells suppressed the growth of indicator cells (Fig. 7B). The CM obtained from the culture in the presence of 10^{-8} to 10^{-11} *M* DP-TAT-59 suppressed the growth of ZR-75-1 cells (Fig. 7C) and MX-1 cells (Fig. 7D) but the CM obtained from cells treated with 4-OH-tamoxifen failed to inhibit the growth of ZR-75-1 cells significantly (Fig. 7C).

To examine the growth-inhibitory potency of some factor from MCF-7 cells, MCF-7 cells and indicator

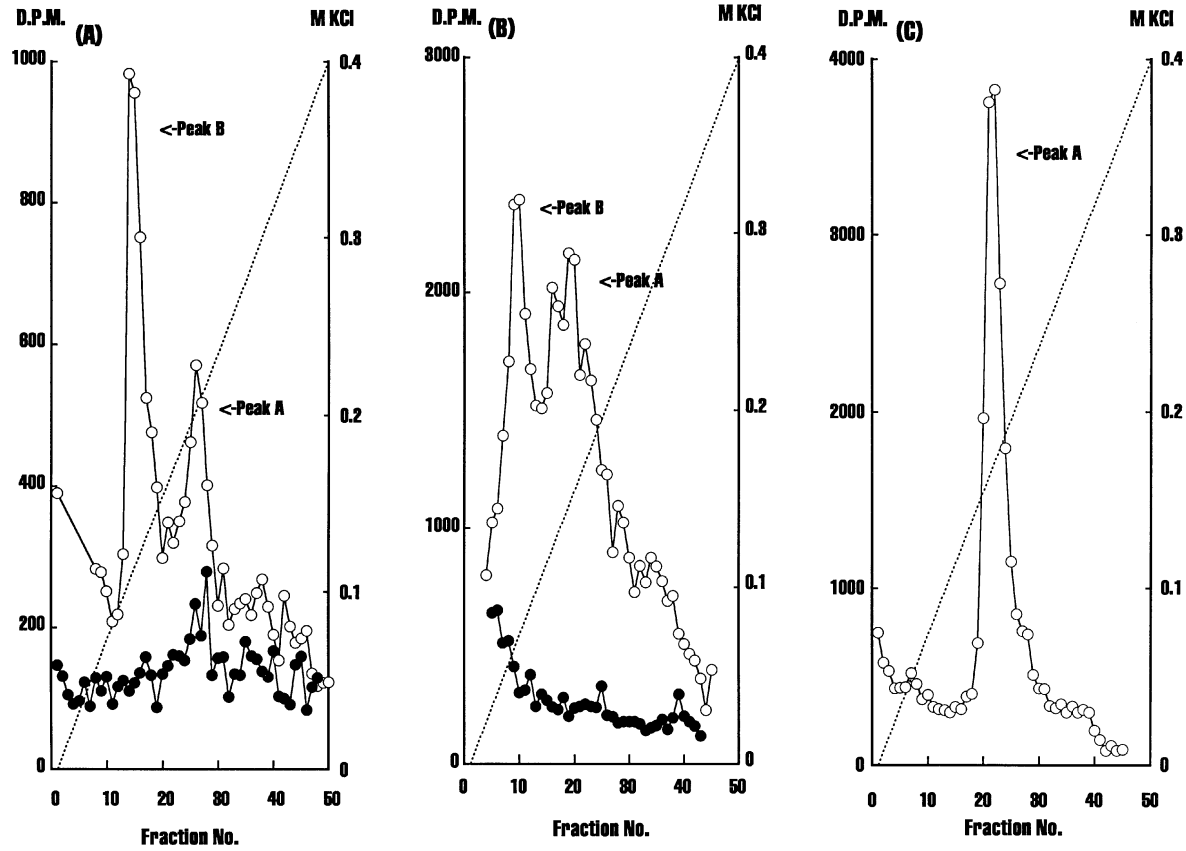


Fig. 6A–C DEAE-Sephadex chromatography of cytoplasmic receptor complexes with [^{3}H]-DP-TAT-59 (**A**), [^{3}H]-4-OH-tamoxifen (**B**) and [^{3}H]-estradiol (**C**). (●—● in the presence of a 200-fold excess of unlabeled estradiol, *dotted line* concentration gradient of KCl)

cells were cocultured, using multiwell plates with culture inserts. The growth of indicator cells was suppressed by cocultured MCF-7 cells treated with antiestrogens (Fig. 8). Treatment with 10^{-7} to 10^{-12} *M* DP-TAT-59 caused stronger suppression of indicator cell growth than did treatment with 4-OH-tamoxifen or tamoxifen. The proliferation rate of indicator cells was not affected by these antiestrogens at the concentrations used in these experiments.

Determination of TGF-β contents in the conditioned medium

As the CM obtained from the cells treated with antiestrogen suppressed the growth of epithelial cell lines and as TGF-β has been reported to inhibit the growth of epithelial cells, the amount of TGF-β in the CM after treatment of MCF-7 cells with antiestrogen was determined (Fig. 9). Treatment with antiestrogen increased the amount of TGF-β in the CM in a dose-dependent manner and the CM after treatment with DP-TAT-59 contained more TGF-β than that with 4-OH-tamoxifen. At a concentration of 10^{-17} *M*, treatment with DP-TAT-59 produced twice the amount of TGF-β than treatment with 4-OH-tamoxifen or tamoxifen. To determine the contribution of TGF-β to the growth-suppressive activity in the CM, the CM was applied to indicator cells in the presence of anti-TGF-β antibody. The growth-suppressive activity of the CM was neutralized by anti-TGF-β antibody (Fig. 10). It is suggested that treatment of MCF-7 cells with DP-TAT-59 produced inhibitory factor(s), which mainly consisted of TGF-β.

Inhibition of production of growth factors from MCF-7 cells

As production of TGF-α is reportedly suppressed by antiestrogens [8], we determined the contents of growth factors in the medium after antiestrogen treatment of MCF-7 cells. As shown in Fig. 11, the concentration of TGF-α in the medium was decreased in a dose-dependent manner after antiestrogen treatment. DP-TAT-59 inhibited the production of growth factor at about a tenfold lower concentration than did 4-OH-tamoxifen.

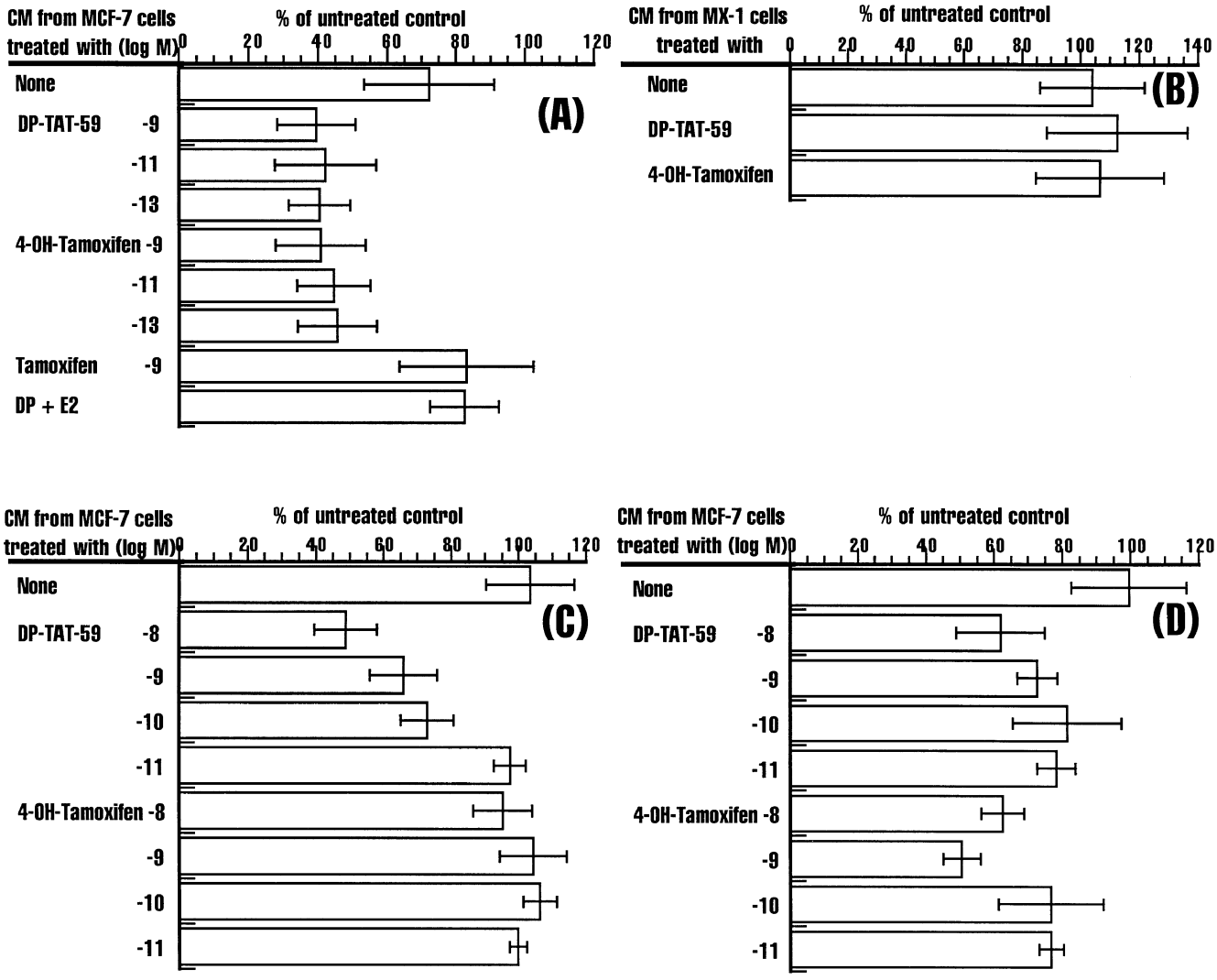


Fig. 7A–C The effect of conditioned medium from MCF-7 cells treated with antiestrogens on the growth of Mv-1-Lu cells (**A**), ZR-75-1 cells (**C**) and MX-1 cells (**D**); **B** the effect of conditioned medium from MX-1 cells (**B**). The bar graphs indicate the means and SD of more than four determinations

Discussion

Comparison of the new antiestrogen DP-TAT-59 with 4-OH-tamoxifen, an active metabolite of the antiestrogen tamoxifen, revealed different inhibitory activities against human mammary carcinoma MCF-7 cells (Fig. 2), despite a similarity in their binding affinities in the whole-cell binding assay (Fig. 5).

Jarman et al. reported that triphenylethylene-type antiestrogens, such as DP-TAT-59 and 4-OH-tamoxifen, do not show high binding capacities on ER in a whole-cell assay system [9]. A similar result was obtained using a short-term incubation system in our experiments (Fig. 3). However, our pharmacokinetic analysis revealed that ER-positive mammary carcinoma cells incorporated these triphenylethylene derivatives at a higher intracellular concentration than estradiol and that the time required for these triphenylethylene derivatives to reach a steady-state intracellular concentration was longer than that for estradiol (Fig. 4). We assume that the amount of cytoplasmic binding sites for these compounds is about four-fold larger than that for estradiol. As ER are reportedly located in the nucleus [13], in the whole-cell assay system, the failure of triphenylethylene derivatives to compete effectively with estradiol may be caused by insufficient concentrations of antiestrogens reaching the nucleus to occupy the ER. Indeed, long-term incubation to equilibrate the concentration of ligand between the intra- and extracellular spaces demonstrated the highly competitive capacity of these antiestrogens in the whole-cell assay (Fig. 5). Therefore, we conclude that these triphenylethylene derivatives kept their binding affinity to ER even in intact cells.

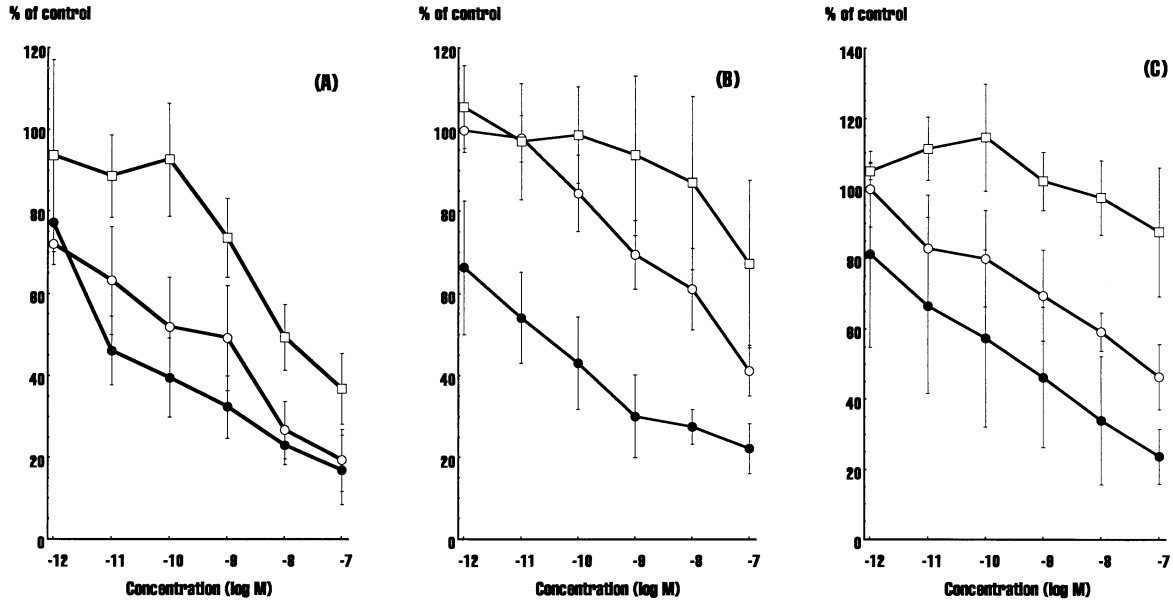


Fig. 8A–C Growth-inhibitory activity of cocultured MCF-7 cells treated with antiestrogens against human mammary carcinoma cell line Mv-1-Lu cells (**A**), ZR-75-1 cells (**B**) and MX-1 cells (**C**). The values shown are the means and SD of more than three determinations (● DP-TAT-59, ○ 4-OH-tamoxifen, □ tamoxifen)

Fig. 9 Concentration of TGF-β produced by MCF-7 cells treated with antiestrogens. The values shown are the means and SD of three determinations (● DP-TAT-59, ■ 4-OH-tamoxifen, □ tamoxifen, △ untreated control)

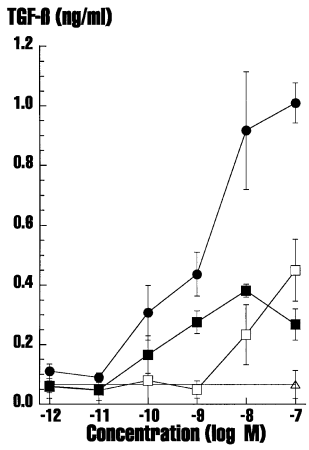
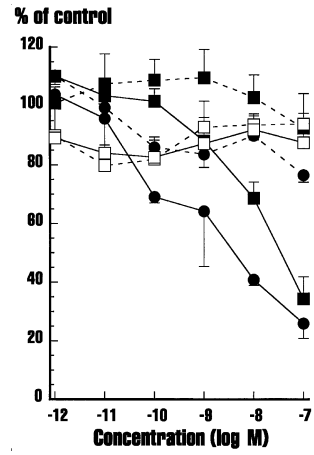


Fig. 10 Neutralization of growth-inhibitory activity of the conditioned medium from MCF-7 cells treated with antiestrogens by 1 μg/ml anti-TGF-β antibody. The *solid lines* represent the growth inhibitory activity in the absence of anti-TGF-β antibody and the *dotted lines* represent the activity in the presence of anti-TGF-β antibody. The values shown are the means and SD of three determinations (● DP-TAT-59, ■ 4-OH-tamoxifen, □ tamoxifen)



H1285, C1628M and 4-OH-tamoxifen, having hormonal activity different from that of estradiol, despite showing an affinity similar to estradiol on their common receptor, have been reported to form a different complex with ER from that of estradiol [11, 20, 22]. The different hormonal activity of these antiestrogens on ER might be caused by a conformational change in the ER complex induced by the antiestrogens. Using ion-exchange column chromatography, we detected a difference in ER complexes not only between antiestrogen and estradiol but also among antiestrogens (Fig. 6). Comparison of DP-TAT-59 with 4-OH-tamoxifen revealed that their complexes formed with ER were quantitatively different, despite the similarity in positive charge on the molecules. The growth inhibitory effect of antiestrogens is related to their affinity for ER [3, 21, 26]. However, DP-TAT-59 suppressed the growth of MCF-7 cells more strongly than 4-OH-tamoxifen, whereas both had similar binding affinity for ER. The inhibitory activity of DP-TAT-59 on the growth of MCF-7 cells did not simply reflect its ability to bind to ER (Fig. 2). However, the exact difference between these two receptor complexes is unclear. The difference between the antiestrogens, in spite of a similar affinity for ER, may contribute to the stronger antagonistic activity of DP-TAT-59 against estrogen-dependent tumors.

Activation of ERs by binding of estrogen might cause expression of various genes which encode enzymes related to DNA synthesis, growth factors or their receptors [1, 6, 7, 10, 30]. The secretion of growth factors which might mainly contribute to tumor growth, is

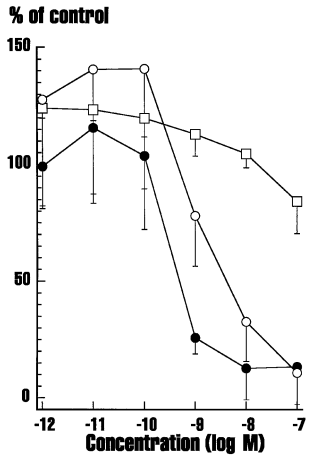


Fig. 11 Inhibition of production of TGF-α from MCF-7 cells by treatment with antiestrogens. The values shown are the means and the SD of three determinations (● DP-TAT-59, ○ 4-OH-tamoxifen, □ tamoxifen)

regulated by estrogens, and they act as auto- or paracrine growth factors [16, 23]. Furthermore, production of TGF-β which is considered as a growth inhibitory factor against mammary tumors is decreased in cells stimulated by estrogens [19]. Since the receptor complexes with DP-TAT-59 were different from those formed with estradiol, the complex with DP-TAT-59 may not activate a gene encoding the production of growth factor or may stimulate a gene that is suppressed by estrogens. In our experiments, the CM from MCF-7 cells treated with DP-TAT-59 showed growth inhibitory activity but that from ER-negative mammary carcinoma MX-1 cells did not (Fig. 7), and after treatment with DP-TAT-59 MCF-7 cells produced TGF-β (Fig. 9) and the secretion of TGF-α was decreased (Fig. 11).

Many breast carcinoma cells remain sensitive to the growth inhibitory effects of TGF-β, suggesting that the local induction of TGF-β could contribute to antitumor efficacy of triphenylethylene derivatives. ER are not uniformly distributed in tumor tissue [18]. Hence ER-negative cells may be stimulated to proliferate by paracrine growth factors secreted from ER-positive cells receiving estrogenic stimulation [17, 31]. Antiestrogens might lower paracrine growth stimulation by ER-positive breast carcinoma cells.

The higher secretion of the growth inhibitory factor, TGF-β, and the stronger suppression of the production of growth-stimulating factors from ER-positive cells following DP-TAT-59 treatment than following 4-OH-tamoxifen or tamoxifen treatment suggests that DP-TAT-59 suppresses the growth of breast cancer more than 4-OH-tamoxifen and tamoxifen. DP-TAT-59 also shows anticancer activity against tumors with low ER levels [27]. We also expect TAT-59 to show sufficient antiestrogenic effect under clinical conditions.

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