when used in combination with other endocrinologically acting drugs with different mechanisms of action.

1. Beatson GT. Suggestions for a new method of treatment with illustrative cases. *Lancet* 1896, ii, 104–107.
2. Santen RJ. Novel methods of oestrogen deprivation for treatment of breast diseases. *Proc R Soc Edinburgh* 1989, **95B**, 255–269.
3. Plowman PN, Walker KJ, Nicholson RI. Remissions of postmenopausal breast cancer during treatment with luteinizing hormone agonist ICI 118630. *Br J Cancer* 1986, **54**, 903–909.
4. Schally AV, Redding TW, Comaru-Schally AM. Potential use of analogues of luteinizing hormone-releasing hormone in the treatment of hormone-sensitive neoplasms. *Cancer Treat Rep* 1984, **68**, 281–289.
5. Santen RJ, Manni A, Harvey H. Gonadotropin releasing hormone (GnRH) analogs for the treatment of breast and prostatic carcinoma. *Breast Cancer Res Treat* 1986, **7**, 129–145.
6. Steele RE, Mellor LB, Sawyer WK, Wasvary JM, Browne LJ. *In vitro* and *in vivo* studies demonstrating potent and selective oestrogen inhibition with the nonsteroidal aromatase inhibitor, CGS 16949A. *Steroids* 1987, **50**, 147–161.
7. Santen RJ, Santen S, Davis S, Veldhuis J, Samojlik E, Ruby E. Aminoglutethimide inhibits extraglanduar oestrogen production in postmenopausal women with metastatic breast carcinoma. *J Clin Endcrinol Metab* 1978, **47**, 1257–1265.
8. Thompson EAJ, Siiteri PK. The involvement of human placental microsomal cytochrome P-450 in aromatization. *J Biol Chem* 1974, **249**, 5373–5378.
9. Dexter RN, Fishman LM, Ney RL, Liddle GW. Inhibition of adrenal corticosteroid synthesis by aminoglutethimide: studies of the mechanism of action. *J Clin Endocrinol Metab* 1967, **27**, 473–480.
10. Brodic AM, Santen RJ. Aromatase in breast cancer and the role of aminoglutethimide and other aromatase inhibitors. *CRC Crit Rev Oncol/Hematol* 1986, 361–396.
11. Schieweck K, Bhatnagar AS, Matter A. CGS 16949A, a new nonsteroidal aromatase inhibitor: effects on hormone-dependent and-independent tumors *in vivo*. *Cancer Res* 1988, **48**, 834–838.
12. Tominaga T, Yoshida Y, Kitamura M, Kosaki G. Effective sequential administration of tamoxifen and medroxyprogesterone acetate for 7,12-dimethylbenz[a]anthracene-induced rat mammary tumors in relation to hormone receptors. *Jpn J Cancer Res* 1985, **76**, 1120–1125.
13. Grodin JM, Siiteri PK, MacDonald PC. Source of oestrogen production in the postmenopausal woman. *J Clin Endocrinol Metab* 1973, **36**, 207–214.
14. Santen RJ, Demers LM, Adlecreutz H *et al.* Inhibition of aromatase with CGS 16949A in postmenopausal women. *J Clin Endocrinol Metab* 1989, **68**, 99–106.
15. Tominaga T, Yoshida Y, Kitamura M, Kosaki G. Effect of combined administration of aminoglutethimide, tamoxifen and medroxyprogesterone acetate on rat mammary tumors induced by 7, 12-DMBA. The 46th Proceedings of the Japan Cancer Association, 1987.

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Interactions between Growth Factor Secretion and Polyamines in MCF-7 Breast Cancer Cells

Fredric J. Cohen, Andrea Manni, Patricia Glikman, Mary Bartholomew and Laurence Demers

Polyamines may be involved in hormone-dependent breast cancer cell proliferation. The antiestrogen 4-hydroxytamoxifen and the polyamine synthesis inhibitor α-difluoromethylornithine (DFMO) inhibited MCF-7 growth, and this effect was additive. Transforming growth factor β (TGF-β) levels were increased by both compounds; again the effect was additive. Exogenous putrescine antagonized DFMO but not the antiestrogen. However, exogenous TGF-β did not inhibit cell growth. Secretion of insulin-like growth factor 1 (IGF-1) was not affected by DFMO-induced polyamine depletion but 4-hydroxytamoxifen increased IGF-1, which suggests an estradiol-like effect. Thus polyamines are involved in basal TGF-β secretion but do not mediate antiestrogen-induced TGF-β secretion. IGF-1 secretion by MCF-7 cells is not under polyamine control. The antiproliferative effects of 4-hydroxytamoxifen and DFMO cannot be accounted for by either suppression of IGF-1 secretion (a growth stimulatory factor) or stimulation of TGF-β production (a growth inhibitory polypeptide).
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INTRODUCTION

STEROID HORMONES may regulate hormone-responsive breast cancer cell proliferation, at least in part, via the production and secretion of various polypeptide growth factors that alter cell growth [1, 2]. Conversely antagonists such as tamoxifen may inhibit breast cancer growth by suppressing hormone-stimulated growth factor production [2] and/or by increasing the secretion of polypeptides, such as transforming growth factor-β (TGF-β), which are thought to inhibit growth effect of breast cancer cells [3]. Putrescine, spermidine, and spermine are essential mediators of hormonally stimulated breast cancer cell growth *in vitro* [4–7] interacting with autocrine/paracrine effectors of such growth [8]. The polyamine pathway may also be involved in antiestrogen-mediated inhibition of breast cancer cell growth *in vitro* [9] and *in vivo* [10].

We have evaluated the role of polyamines in antiestrogen-regulated secretion of insulin-like growth factor 1 (IGF-1) and TGF-β by MCF-7 breast cancer cells in culture.

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MATERIALS AND METHODS

Chemicals

4-hydroxytamoxifen was provided by ICI. α-Difluoromethylornithine (DMFO) was supplied by Merrell Dow. Putrescine dihydrochloride, estradiol-17-β (E_2), and aprotinin (12 TIU/mg protein) were purchased from Sigma. hTGF-β1 (crude and purified), [^{125}I]hTGF-β (4.07 MBq/mg) and protein-A purified, rabbit IgG fraction anti-TGF-β were purchased from R&D Systems, Minneapolis. Richter's improved minimal essential medium (IMEN) was purchased from Grand Island Biological. Fetal bovine serum (FBS) was purchased from Irvine Scientific.

Cells

MCF-7 and MDA-MB-231 human breast cancer cells were provided by Dr M.E. Lippman (NIH, Bethesda). They were maintained in 75 cm^2 flasks (Costar) containing putrescine-free IMEM with phenol red and 10% FBS in a humidified atmosphere of 5% CO_2 in air at 37°C.

Growth studies and collection of conditioned media

Logarithmically growing MCF-7 breast cancer cells were harvested with 0.25% trypsin/EDTA solution and plated in 100 mm dishes (Falcon) at 4 × 10^5 cells per dish (four dishes per experimental condition) in IMEM containing phenol red and 2% FBS. After 24 h to allow for attachment, medium was discarded and fresh medium containing the appropriate treatments or control was added (day 0). 4-OH-tamoxifen and E_2 were dissolved in 100% ethanol before addition to the dishes (final ethanol concentration in all dishes was 0.1% or under). This procedure was repeated 48 h later. On day 4, the cells were washed for 20 min with phosphate-buffered saline (PBS) and cultured under continuous treatment for 20–24 h in serum-free IMEM (supplemented with 4 mmol/l glutamine, 2 μg/ml transferrin, 1 μg/ml fibronectin, 4 g/l bovine serum albumin-[fraction V, BSA], 20 mmol/l 'hepes' buffer, and phenol red). The conditioned medium was discarded and fresh supplemented serum-free IMEM containing the experimental treatments was added. Conditioned media were collected 48 h later and 0.1% aprotinin (12 TIU/mg protein, Sigma) was added immediately. The samples were kept on ice until they could be processed further.

The cells were harvested by brief trypsinization and then suspended in L-15 medium (Gibco). Cell suspensions were divided into two: one portion was immediately placed on ice until it could be processed for polyamine measurement; the other was used for cell counting by hemocytometer.

Growth study with anti-TGF-β

Logarithmically growing MCF-7 cells were plated in 24-well dishes (Falcon) at 6 × 10^4 cells per well (three wells per experimental condition) in IMEM containing 2% FBS and phenol red. Cells were allowed 24 h for attachment (day 0) and were then cultured in serum-free conditions as before. Media containing appropriate treatments were added on days 0 and 2. Cells were harvested and counted on day 4. Because of the high cost and limited availability of anti-TGF-β antibody (R&D Systems, Minneapolis), this experiment was done only once.

Processing of cell pellets and polyamine measurement

After a PBS wash, cells were suspended in 0.6 mol/l $HClO_4$ to give a final cell concentration between 1 and 2 × 10^7/ml. The cell extracts were stored at −70°C until assay. Polyamines were measured by fluorimetry [9]. We also calculated the spermidine:spermine ratio which correlates with cellular proliferative activity [11].

Processing of conditioned media and assay for secreted TGF-β

Conditioned media were centrifuged at 800 *g* for 10 min at 4°C, clarified through a 0.4 μm filter (Corning), and concentrated approximately 4-fold in an ultrafiltration cell with a 5000 molecular weight cutoff ('YM5', Amicon). Although not measured here, recovery of [^{125}I]hTGF-β added immediately after collection was higher than 70% under nearly identical conditions [3]. Conditioned media concentrates were frozen at −70°C.

Most cell lines tested [12, 13], including MCF-7 cells [3], secrete at least a fraction of TGF-β as a biologically inactive form that is not recognized by the TGF-β radioreceptor assay. Consequently, to measure total TGF-β secretion, we activated any latent TGF-β in the conditioned media by transient acidification before assay. TGF-β loss due to thawing and re-freezing has not been reported; on the contrary, this treatment seems to activate latent TGF-β [14]. Samples were thawed and acidified with 5 mol/l HCl followed by 5 min at room temperature and reneutralization with 5 mol/l NaOH. The conditioned media samples were frozen at −70°C and lyophilized.

To assay TGF-β [15] 1.2 × 10^5 MDA-MB-231 cells per well were plated in 24-well plates (Falcon) in 0.5 ml IMEM supplemented with 10% FBS. 48 h later, the confluent monolayers were washed three times with binding buffer (0.1% BSA, 25 mmol/l hepes, IMEM, pH 7.4) and incubated for 2 h at room temperature with 50 pmol/l [^{125}I]TGF-β and increasing concentrations of unlabelled TGF-β (2–20,000 pmol/l) in 0.2 ml buffer. After incubation, the cells were washed three times with ice-cold PBS/0.1% BSA and solubilized with 'Triton X-100' solution (1% Triton X-100, 25 mmol/l hepes, 10% glycerol, 1 mg/ml BSA, pH 7.5). Cell-associated radioactivity was measured in a gamma counter. The concentration of TGF-β in conditioned media was obtained from a standard curve. All assays were from duplicate wells.

Radioimmunoassay for secreted IGF-1

Samples of the conditioned media processed for TGF-β assay were used for measurement of IGF-I in a somatomedin-C kit (Nichols Institute Diagnostics, San Juan Capistrano, California) according to the manufacturer's instructions [16]. We did not use acid–ethanol extraction [2].

Measurement of TGF-β receptors

Breast cancer cells (2 × 10^5 MCF-7 or 1.2 × 10^5 MDA-MB-231 cells per well) were plated in 24-well plates in IMEM supplemented with 2% FBS. 48 h later the medium was changed to IMEM with 0.5% FBS. After overnight incubation, cells were treated for 8 min at 4°C with 1% acetic acid, 0.9% NaCl, 2 mg/ml BSA (pH 3.7) to remove endogenous TGF-β bound to its receptor [17]. After three washes with binding buffer, the monolayers were incubated in triplicate wells with 50 pmol/l [^{125}I]TGF-β plus increasing concentrations (2–3846 pmol/l) of unlabelled TGF-β in a total volume of 0.2 ml buffer for 4 h at 0–4°C. The cells were washed three times with ice-cold PBS/0.1% BSA and solubilized for 90 min at 37°C with 0.5 ml prewarmed Triton X-100 solution [3].

Statistical methods

Two-factor analysis of variance was used for testing differences among treatments followed when significant by the Student–Newman–Keuls procedure. Dependent variables analyzed in this way were: cell growth as a percentage of control, cellular polyamine levels, and secreted growth factor activity. The log transformation was applied to stabilize the variances for all dependent variables other than cell growth and IGF.

RESULTS

Treatment effects on cell growth

4-OH-tamoxifen and DFMO had a similar antiproliferative effect, reducing mean cell number to 32.2 (SE 0.75) and 26.5 (2.4) % of control, respectively (Fig. 1A). The two drugs combined induced a significant further reduction (Fig. 1A, B). Putrescine reversed the antiproliferative effect of DFMO but not that of 4-OH-tamoxifen (Fig. 1B) which could, however, be reversed by E_2 (Fig. 1C). The effect of E_2 was blocked in a dose-dependent fashion by DFMO (Fig. 1C). 100 pmol/l TGF-β had only a modest antiproliferative effect (77.2 [8.2]% of control) (Fig. 1A). In agreement with our previous observation [9] putrescine alone did not significantly influence MCF-7 cell proliferation (Fig. 1B). The lack of stimulatory effect of E_2 alone was not surprising (Fig. 1C) since the experiments were done in of phenol red and partly under serum-repleted conditions.

Treatment effects on cellular polyamine levels

4-OH-tamoxifen modestly reduced spermidine level and the spermidine:spermine ratio (Tables 1–3). When we combined all the experimental conditions (n = 9) in which 4-OH-tamoxifen was added alone, drug effects on cellular spermidine levels ($P < 0.001$) and the spermidine:spermine ratio ($P < 0.01$) were significant. Both the suppression of the cellular levels of spermidine and the spermidine:spermine ratio induced by 4-OH-tamoxifen were reversed by E_2 (Table 3). E_2 alone caused a significant rise in putrescine and spermidine levels as well as in the spermidine:spermine ratio (Table 3). As expected, the administration of DFMO either alone or in combination suppressed in a dose-dependent fashion cellular levels of putrescine and spermidine as well as the spermidine:spermine ratio. Exogenous putrescine repleted cellular polyamine pools to levels in excess of control for putrescine (Table 2). Administration of TGF-β did not influence any of the polyamines (Table 1).

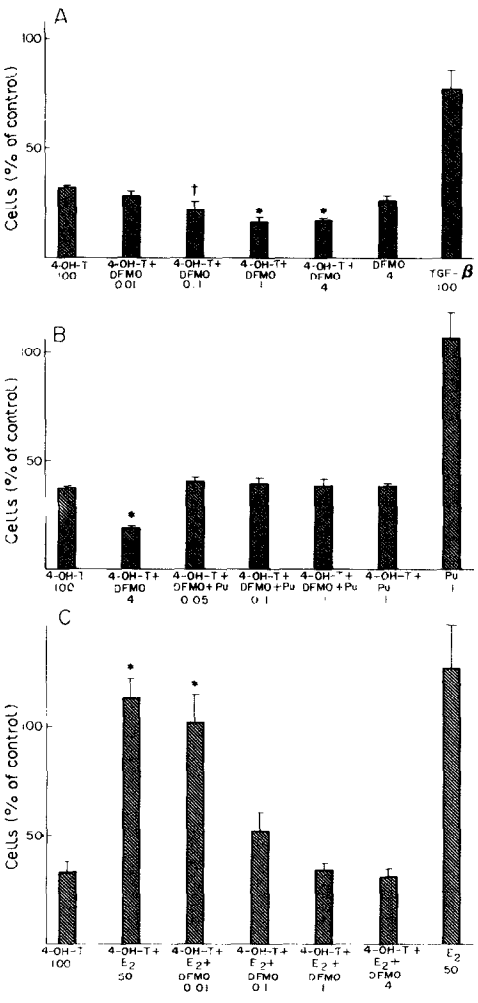

Fig. 1. Treatment effects on MCF-7 breast cancer cell proliferation. Mean (SE), n = 3. A, B, and C correspond to Tables 1–3. 4-OH-T=4-OH-tamoxifen in nmol/l and PU = putrescine. PU and DFMO in mmol/l; E_2 in nmol/l. α = 0.05: A— *vs. other groups and † vs. 4-OH-T; B— *vs. other groups, and C— *vs. other groups except E_2.

*Table 1. Effect of 4-OH-tamoxifen (T) DFMO (D), or TGF-β on cellular polyamine levels in MCF-7 breast cancer cells**

Test	Polyamines (nmol/10^6 cells) PU	SP	SM	SP:SM
1 Control	0.79 (0.09)	1.27 (0.08)	1.29 (0.06)	0.98 (0.02)
2 T	0.79 (0.08)	0.97 (0.06)	1.35 (0.01)	0.72 (0.04)*
3 T + D (0.01)	0.34 (0.14)*	0.63 (0.19)*	1.60 (0.16)	0.39 (0.10)*
4 T + D (0.1)	ND†	0.03 (0.03)†	1.52 (0.21)	0.02 (0.02)†
5 T + D (1)	ND†	ND†	2.04 (0.22)	0†
6 T + D (4)	ND†	ND†	1.45 (0.26)	0†
7 D (4)	0.08 (0.07)†	0.10 (0.10)†	1.25 (0.33)	0.05 (0.05)†
8 TGF-β	0.93 (0.02)	1.39 (0.06)	1.24 (0.16)	1.18 (0.24)

Mean (SE), n = 3.
4-OH-T 100 nmol/l. DFMO in mmol/l; TGF-β 100 pmol/l. ND = not detectable. PU = putrescine, SP = spermidine, and SM = spermine. P = 0.05: *vs. other tests and †vs. tests 1–3 and 8.

Table 2. Effect of 4-OH-tamoxifen, putrescine, or DFMO

Test	Polyamines (nmol/10^6 cells) PU	SP	SM	SP:SM
1 Control	0.57 (0.03)	1.12 (0.09)	0.86 (0.13)	1.33 (0.10)
2 T	0.42 (0.02)	0.65 (0.04)	0.66 (0.10)	1.01 (0.11)
3 T + D	ND*	ND*	1.05 (0.20)	0*
4 T + D + PU (0.01)	1.09 (0.05)‡	0.74 (0.05)	0.73 (0.14)	1.06 (0.16)
5 T + D + PU (0.1)	1.27 (0.11)‡	0.67 (0.05)	0.64 (0.10)	1.08 (0.12)
6 T + D + PU (1)	2.43 (0.25)†	0.73 (0.07)	0.67 (0.14)	1.16 (0.18)
7 T + PU (4)	2.84 (0.43)†	1.05 (0.14)	0.73 (0.18)	1.70 (0.61)
8 PU (1)	2.23 (0.20)†	1.54 (0.42)§	0.77 (0.004)	2.00 (0.56)§

DFMO 4 mmol/l, and putrescine in mmol/l. $P = 0.05$: *vs. other tests, †vs. 1–5, ‡vs. 1–3 and 6–8, and §vs. 2–6.

Table 3. Effect of 4-OH-T and/or E_2 with and without DFMO

Tests	Polyamines (nmol/10^6 cells) PU	SP	SM	SP:SM
1 Control	0.36 (0.01)	1.02 (0.08)	0.84 (0.13)	1.20 (0.06)
2 T	0.34 (0.04)	0.65 (0.04)*	0.85 (0.06)	0.77 (0.02)‡
3 T + E_2 (50)	0.33 (0.01)	1.05 (0.03)	0.84 (0.04)	1.25 (0.04)
4 T + E_2 + D (0.01)	0.16 (0.02)*	0.89 (0.09)	1.08 (0.09)	0.82 (0.03)‡
5 T + E_2 + D (0.1)	ND†	ND†	0.71 (0.01)	0†
6 T + E_2 + D (1)	ND†	ND†	0.65 (0.06)	0†
7 T + E_2 + D (4)	ND†	ND†	0.72 (0.09)	0†
8 E_2 (1)	0.84 (0.48)*	1.34 (0.11)*	0.90 (0.05)	1.48 (0.06)*

E_2 mmol/l. $P = 0.05$: *vs. other tests, †vs. 1–4 and 8, and ‡vs. 1 and 3.

Treatment effects on growth factor production

4-OH-tamoxifen caused an increase in TGF-β secretion ranging between 1.4-fold and 1.8-fold. When we combined all the experimental conditions in which 4-OH-tamoxifen was added alone, the stimulatory effect on TGF-β secretion was significant ($P < 0.0027$). The rise in TGF-β secretion induced by 4-OH-tamoxifen was similar to that observed with DFMO (1.8-fold, Fig. 2A). Treatment with both compounds induced a further increase in TGF-β secretion (between 2.7-fold and 3.0-fold). Putrescine reversed the DFMO but not the 4-OH-tamoxifen induced changes in TGF-β secretion (Fig. 2B). E_2, alone or in combination with 4-OH-tamoxifen, did not influence TGF-β secretion (Fig. 2C). On the other hand, E_2 prevented the rise in TGF-β levels with the combined administration of 4-OH-tamoxifen and DFMO (Fig. 2A, B).

Surprisingly, 4-OH-tamoxifen induced a consistent and significant increase in IGF-I secretion ranging between 1.8-fold and 2.7-fold. Such stimulation was similar to that induced by E_2 (Fig. 2C). A further increase in IGF-I level was observed with 4-OH-tamoxifen plus E_2 (Fig. 2C). Polyamine depletion induced by DFMO did not influence either basal (Fig. 2A) or stimulated (Fig. 2A–C) IGF-I production. Exogenous TGF-β administration also did not significantly affect IGF-1.

Anti-TGF-β and 4-OH-tamoxifen inhibition

Anti-TGF-β polyclonal antibody (100 μg/ml) failed to influence either basal or 4-OH-tamoxifen-inhibited (100 nmol/l) MCF-7 cell proliferation (data not shown). In addition, administration of either 100 or 500 pmol/l TGF-β simultaneously tested did not inhibit cell growth (data not shown). In agreement with our inability to demonstrate a biological effect of TGF-β we could not detect TGF-β receptors in our MCF-7 cells, although these receptors were present in the hormone-independent MDA-MB-231 breast cancer cell line as established by competitive binding of radiolabelled TGF-β (data not shown).

DISCUSSION

Our results showed that polyamine depletion induced by DFMO was as effective as 4-OH-tamoxifen in inhibiting proliferation and in increasing the secretion of TGF-β. The specificity of the DFMO effect through the polyamine pathway was supported by the ability of exogenous putrescine to prevent the DFMO-induced rise in TGF-β production (Fig. 2B) as well as inhibition of proliferation (Fig. 1B).

The degree of stimulation by 4-OH-tamoxifen of TGF-β production observed in our experiments (about 1.8-fold) was less than that reported (3–4-fold) [3] with acid extraction Nevertheless antiestrogen-induced rises in TGF-β were significant in combined analysis. 4-OH-tamoxifen lowered polyamine levels, although less so than we found before [9]. This suppressive effect, however, was significant in combined analysis. Such polyamine suppression may mediate antiestrogen-induced stimulation of TGF-β secretion, although this is unlikely for the following reasons. First, 4-OH-tamoxifen stimulated TGF-β

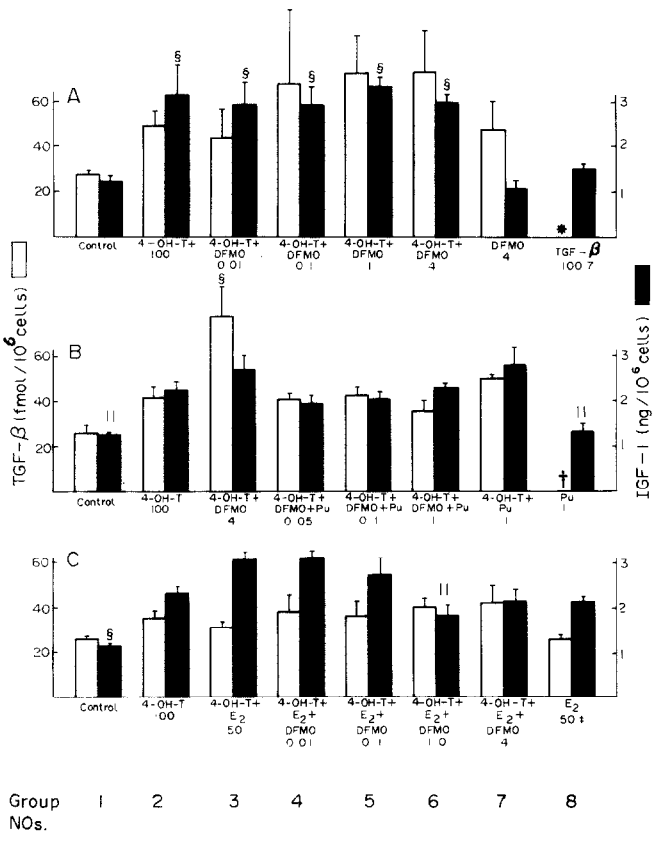

Fig. 2. Treatment effects on growth factor secretion, mean (S.E.), $n = 3$. *TGF-β not measured since TGF-β added to media. † $n = 1$ (55.11 fmol/10⁶ cells). ‡ $n = 2$, $\alpha = 0.05$: A—§ vs. groups 1, 7, and 8; B—§ vs. 1 and 2 and 4–6, ‖ vs. 2–7; and C—§ vs. other groups and ‖ vs. 3 and 4.

production similarly to DFMO while having a smaller effect on polyamine pools. Secondly, 4-OH-tamoxifen plus DFMO stimulated TGF-β production more than the individual treatments, suggesting that the two drugs may work through different mechanisms. Thirdly, repletion of polyamine pools with exogenous putrescine (Table 2) did not prevent the 4-OH-tamoxifen-induced increase in TGF-β secretion (Fig. 2B). Addition of exogenous putrescine also did not reverse the antiproliferative effect of 4-OH-tamoxifen (Fig. 1B). This finding agrees with our previous observations [9] and, with the present data, suggests that polyamines are not primarily involved in either the antiproliferative action of 4-OH-tamoxifen or in antiestrogen-induced TGF-β secretion. Surprisingly, E_2 either alone or in the presence of 4-OH-tamoxifen did not suppress TGF-β secretion (Fig. 2C), as reported by Knabbe *et al.* [3]. However, E_2 did prevent the rise in TGF-β levels induced by 4-OH-tamoxifen plus DFMO (Fig. 2C).

TGF-β did not mediate the antiproliferative effect of 4-OH-tamoxifen or DFMO since our MCF-7 cells did not contain TGF-β receptors and were not growth inhibited by exogenous TGF-β. Our findings, while at variance with Knabbe *et al.* [3], agree with Arteaga *et al.* [15] that four estrogen receptor positive breast cancer cell lines (including MCF-7 cells) lacked TGF-β receptors and were unresponsive to exogenous TGF-β. Nevertheless, it is likely that TGF-β produced by hormone responsive cells has important paracrine effects *in vivo* on neighboring hormone-resistant cells since estrogen receptor negative cell lines contain TGF-β receptors and are growth inhibited by this polypeptide [3, 15]. Furthermore TGF-β production by hormone-responsive and resistant cells may have additional complex paracrine effects on breast cancer growth *in vivo* by stimulating the proliferation and growth factor secretion of surrounding fibroblasts and other mesenchymally derived cell types [18, 19].

IGF-1 immunoactivity in the conditioned media of breast cancer cell lines, has not been fully defined. Although IGF-I mRNA has been shown by northern blotting [3], a specific ribonuclease protection assay failed to show in human breast cancer cell lines [20]. Thus, rather than true IGF-1, an IGF-1 related peptide might be being measured by the radioimmunoassay. Part of the IGF-1 immunoactivity may be accounted for by IGF-1 binding protein(s) produced by these cell lines [21, 22] and which can interfere with the assay [23]. In any event, immunoactive IGF-1 secretion, previously shown to be stimulated by E_2 and inhibited by antiestrogens, is tightly coupled to growth regulation by these agents, thus suggesting a potential role for this growth factor in the autocrine/paracrine control of breast cancer cell proliferation by estrogens and antiestrogens [2]. In contrast, and unexpectedly, we found that 4-OH-tamoxifen, while exerting a potent antiproliferative effect, stimulated IGF-1 production to the same degree as E_2 itself (Fig. 2). Furthermore, 4-OH-tamoxifen plus E_2 additionally increased in IGF-1 secretion (Fig. 2C). We suspect that this finding represents an estrogen agonistic effect of 4-OH-tamoxifen [24–26]. Polyamine depletion induced by DFMO failed to influence basal and 4-OH-tamoxifen stimulated IGF-1 secretion in agreement with our preliminary finding suggesting lack of polyamine involvement in E_2 regulated IGF-1 production [27].

Thus our data suggest that polyamines are involved in basal TGF-β secretion but do not mediate antiestrogen-induced TGF-β secretion or inhibition of proliferation. In addition, immunoactive IGF-1 secretion by MCF-7 breast cancer cells is not under polyamine control. Neither growth factor mediates the antiproliferative effect of 4-OH-tamoxifen or DFMO. Our results do not exclude a role for either growth factor in the autocrine/paracrine control of breast cancer growth. Finally our data confirm reports [28, 29] of a potent antiproliferative effect of DFMO on human mammary tumor cells in culture. This effect is similar to and at least additive to that of tamoxifen.

1. Bates SE, Davidson NE, Valverius EM *et al.* Expression of transforming growth factor and its messenger ribonucleic acid in human breast cancer: its regulation by estrogen and its possible functional significance. *Mol Endocrinol* 1988, **2**, 543–555.
2. Huff KK, Knabbe C, Lindsey R *et al.* Multihormonal regulation of insulin-like growth factor-1-related protein in MCF-7 human breast cancer cells. *Mol Endocrinol* 1988, **2**, 200–208.
3. Knabbe C, Lippman ME, Wakefield LM *et al.* Evidence that transforming growth factor-β is a hormonally regulated negative growth factor in human breast cancer cells. *Cell* 1987, **48**, 417–428.
4. Manni A, Badger B, Wright C, Ahmed SR, Demers LM. Effect of progestins on growth of experimental breast cancer in culture: interaction with estradiol and prolactin and involvement of the polyamine pathway. *Cancer Res* 1987, **47**, 3066–3071.
5. Hoggard N, Green CD. Polyamines and growth regulation of cultured human breast cancer cells by 17β-estradiol. *Mol Cell Endocrinol* 1986, **46**, 71–78.
6. Kendra KL, Katzenellenbogen BS. An evaluation of the involvement of polyamines in modulating MCF-7 human breast cancer cell proliferation and progesterone receptor levels by estrogen and antiestrogen. *J Steroid Biochem* 1987, **28**, 123–128.

7. Lima G, Shiu RPC. Role of polyamines in estradiol-induced growth of human breast cancer cells. *Cancer Res* 1985, **45**, 2466–2470.
8. Manni A, Wright C, Demers L, Bartholomew M. Polyamines and autocrine control of *N*-nitrosomethylurea-induced rat mammary tumor growth *in vitro* by progesterone. *Cancer Res* 1988, **48**, 3058–3061.
9. Cohen FJ, Manni A, Glikman P, Bartholomew M, Demers L. Involvement of the polyamine pathway in antiestrogen-induced growth inhibition of human breast cancer. *Cancer Res* 1988, **48**, 6819–6825.
10. Manni A, Wright C, Demers L, Luk G. Polyamines and hormonal regulation of *N*-nitrosomethylurea-induced rat mammary tumor growth *in vivo*. *Cancer Res* 1986, **46**, 4938–4941.
11. Russell DH, McVicker TA. Polyamine biogenesis in the rat mammary gland during pregnancy and lactation. *Biochem J* 1972, **130**, 71–76.
12. Lawrence DA, Pircher R, Kryceve-Martinereie, Lullien P. Normal embryo fibroblasts release transforming growth factors in a latent form. *J Cell Physiol* 1984, **121**, 184–188.
13. Coffey RJ Jr, Shipley GD, Moses HL. Production of transforming growth factors by human colon cancer cell lines. *Cancer Res* 1986, **46**, 1164–1169.
14. Lyons RM, Keski OJ, Moses HL. Proteolytic activation of latent transforming growth factor-β from fibroblast-conditioned medium. *J Cell Biol* 1988, **106**, 1659–1665.
15. Arteaga CL, Tandon AK, VonHoff DD, Osborne CK. Transforming growth factor-β: potential autocrine growth inhibitor of estrogen receptor-negative human breast cancer cells. *Cancer Res* 1988, **48**, 3898–3904.
16. Huff KK, Kaufman D, Gabbay KH, Spencer EM, Lippman ME, Dickson RB. Secretion of an insulin-like growth factor-1-related protein by human breast cancer cells. *Cancer Res* 1986, **46**, 4613–4619.
17. Massague J, Kelly B. Internalization of transforming growth factor-β and its receptor in BALB/c 3T3 fibroblasts. *J Cell Physiol* 1986, **128**, 216–222.
18. Tucker RF, Volkenant ME, Branum EL, Moses HL. Comparison of intra- and extracellular transforming mouse embryo cells. *Cancer Res* 1983, **43**, 1581–1586.
19. Moses HL, Coffey RJ, Leof EB, Lyons RM, Keski-Oja J. Transforming growth factor-β regulation of cell proliferation. *J Cell Physiol* 1987, **5**, 1–7.
20. Yee D, Paik S, Lebovic GS *et al.* Analysis of insulin-like growth factor-1 gene expression in malignancy: evidence for a paracrine role in human breast cancer. *Mol Endocrinol* 1989, 509–517.
21. De Leon DD, Bakker B, Wilson DM, Hintz RL, Rosenfeld RG. Demonstration of insulin-like growth factor (IGF-I and II) receptors and binding protein in human breast cancer cell lines. *Biochem Biophys Res Commun* 1988, **152**, 398–405.
22. Yee D, Favoni RE, Lupu R. The insulin-like growth factor binding protein BP-25 is expressed by human breast cancer cells. *Biochem Biophys Res Commun* 1989, **158**, 38–44.
23. Daughaday WH, Kapadia M, Mariz I. Serum somatomedin binding proteins: physiologic significance and interference in radiology and assay. *J Lab Clin Med* 1987, **109**, 355–363.
24. Vignon F, Bouton MM, Rochefort H. Antiestrogens inhibit the mitogenic effect of growth factors on breast cancer cells in the total absence of estrogens. *Biochem Biophys Res Commun* 1987, **146**, 1502–1508.
25. Horwitz KB, McGuire WL. Endocrine treatment in breast cancer. In: Hemminsen B, Linder F, Steichele C, eds. *A New Approach of Endocrine Treatment*. New York, Springer, 1984, 45–48.
26. Baulieu EE. A rationale for combined antiestrogen plus progestin administration in breast cancer. In: Camio L, Robustelli della Cuna G, Taylor RW, eds. *Role of Medroxyprogesterone in Endocrine-related Tumors*. New York, Raven Press, 1983, Vol. 2, 15–18.
27. Glikman P, Manni A, Bartholomew M, Demers L. Polyamine involvement in insulin-like growth factor-1 secretion and action in human breast cancer. Proceeding 80th Annual Meeting American Association for Cancer Research, 1989, Vol. 30, 73 (Abstr #289).
28. Glikman PL, Manni A, Bartholomew M, Demers L. Polyamine involvement in the growth of hormone-responsive and resistant human breast cancer cells in culture. *Cancer Res* 1989, **49**, 1371–1376.
29. Thomas T, Kiang, DT. Additive growth-inhibitory effects of DL-α-difluoromethylornithine and antiestrogens on MCF-7 breast cancer cell line. *Biochem Biophys Res Commun* 1987, **148**, 1338–1345.

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Lack of binding of gestodene to estrogen receptor in human breast cancer tissue

K. Pollow, M. Juchem, H.J. Grill, W. Elger, S. Beier, K. Schmidt-Gollwitzer and B. Manz

Competition studies with progesterone and estradiol receptors of human myometrial tissue as well as of mammary cancer tissue showed that gestodene bound with high affinity to the progesterone receptor, as did other synthetic and natural progestogens. However, gestodene did not bind to the estradiol receptor. The relative binding affinities of all tested synthetic and natural ligands showed no organ-specific differences and no differences between neoplastically transformed and normal tissues.
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INTRODUCTION

GESTODENE, a 19-nortestosterone derivative, is an effective progestogen [1]. Iqbal *et al.* [2, 3] reported significant binding of gestodene to the estradiol receptor of human malignant tissue but no binding to this receptor in normal breast tissue or endometrium. Such findings are surprising because they demonstrate that a progestogen such as gestodene, which is structurally related to levonorgestrel and its optical isomer *d*-norgestrel, displaces estradiol from its receptor. Therefore, we have investigated the binding of gestodene to the estradiol receptor of normal and neoplastic breast tissue as well as that of normal myometrium.