Estrogen- and Growth Factor-Induced Proliferation of MCF-7 Cells: Inhibition by 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)

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Abstract

The effects of 17β-estradiol (E2), epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1), and transforming growth factor α (TGFα) (1 and 10 nM) on proliferation of MCF-7 human breast cancer cells were investigated. Treatment with 10 nM E2, EGF or TGFα resulted in 3- to 5-fold increase in cell growth, whereas a 20-fold increase was observed after treatment with 10 nM IGF-1. In cells cotreated with E2 or the growth factors plus 10 nM TCDD, there was approximately a 50% decrease in the proliferative response for all the mitogens. Thus, TCDD exhibited both antiestrogenic and antimitogenic activity in MCF-7 cells.

Introduction

Kociba and coworkers (1) first reported that female Sprague-Dawley rats maintained on TCDD in the diet (0.1, 0.01 and 0.001 μg/kg/day) for 2 years exhibited a dose-dependent decrease in spontaneous mammary and uterine tumors. Subsequent studies have demonstrated that TCDD also inhibited carcinogen-induced mammary tumor formation and growth in rats and mammary tumor growth in immune-deficient mice bearing MCF-7 human breast cancer cell xenografts (1-3). The antiestrogenic activity of TCDD and related AhR agonists has been extensively investigated *in vivo* in the female rodent uterus and *in vitro* in human breast cancer cells (reviewed in 4). For example, TCDD inhibited the following E2-induced responses in MCF-7 or T47D human breast cancer cells: cell proliferation; [^{3}H]thymidine uptake; postconfluent focus production; secretion of pS2, cathepsin D, procathepsin D and tissue plasminogen activator activity; PR binding; and ER,PR, pS2, prolactin receptor and cathepsin D gene expression. Moreover, using estrogen-responsive promoter-reporter constructs derived from the 5'-regions of the pS2 and cathepsin D genes, TCDD also inhibited E2-induced reporter gene activity in transiently transfected MCF-7 cell (5,6). The antiestrogenic activities of TCDD resembled those reported for "pure" antiestrogens such as ICI 164,384 which act through the ER, and mechanistic studies have uncovered some unique pathways which govern interactions between the ER and AhR. Research in this laboratory and others have demonstrated that for at least three E2-responsive genes, Hsp 27, CD and pS2, inhibition of E2-induced transactivation is related to direct interaction of the nuclear AhR complex with inhibitory DRE (iDRE) sequences in the 5'-promoter region of these genes (5,6). Previous studies in this laboratory have shown that insulin, insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF), and transforming growth factor α (TGFα) induce growth of MCF-7

or T47D human breast cancer cells in culture and TCDD partially inhibits some of these responses (7-9). The present study compares the proliferative activities of 17β-estradiol (E2), TGFα, EGF and IGF-1 alone on the growth of MCF-7 cells and the antimitogenic activity of TCDD in cells cotreated with E2/growth factors plus TCDD.

Materials and Methods

Cell Culture. MCF-7 cells were obtained from the American Type Culture Collection (ATCC) and grown in MEM supplemented with 10% fetal bovine serum plus $NaHCO_3$ (2.2 g/L), gentamycin (2.5 mg/L), penicillin/streptomycin (10,000 units/L and 10 mg/L), amphotericin B (1.25 mg/L) and insulin (10 mg). Cells were maintained in 150 cm^2 tissue culture flasks/plates and incubated at 37°C in a humidified mixture of 5% CO_2 and 95% air under atmospheric pressure.

Cell Proliferation Assay. Cells were seeded at 7.5 x 10^4 cells/well in 6-well plates in media containing 2 ml DME/F12 without phenol red, supplemented with 5% FBS treated with dextran-coated charcoal (FBS-DCC). After 24 hr, the media was changed to serum free and the cells were treated with E2, growth factors, or growth factors plus TCDD for 11 days. The medium was changed and cells were redosed every 48 hr. The cells were harvested and counted using a Coulter Z1 cell counter. All determinations were carried out in triplicate, and results are expressed as means ± SD.

Results and Discussion

The results summarized in Figure 1 show that E2, EGF, TGFα and IGF-1 induce proliferation of MCF-7 cells in culture. Ten nM concentrations of E2, EGF and TGFα induce a 3- to 5-fold increase in MCF-7 cell proliferation; however, 10 nM IGF-1 induced a 20-fold increase and was the most potent mitogen for this response. In cells cotreated with TCDD plus E2, there was a significant decrease in E2-induced cell proliferation and this was consistent with the antiestrogenic activity of TCDD. The results of this study confirmed that TCDD also inhibited growth factor (GF)-induced proliferation of MCF-7 cells and these data were consistent with previously reported data (7-9). The mechanisms of growth factor-mediated effects in breast cancer cells are complex and may involve activation of the estrogen receptor via protein kinase-dependent phosphorylation pathways. Thus, it is also possible that the antimitogenic activity of TCDD may involve both ER-dependent and -independent pathways and these are currently being investigated in this laboratory.

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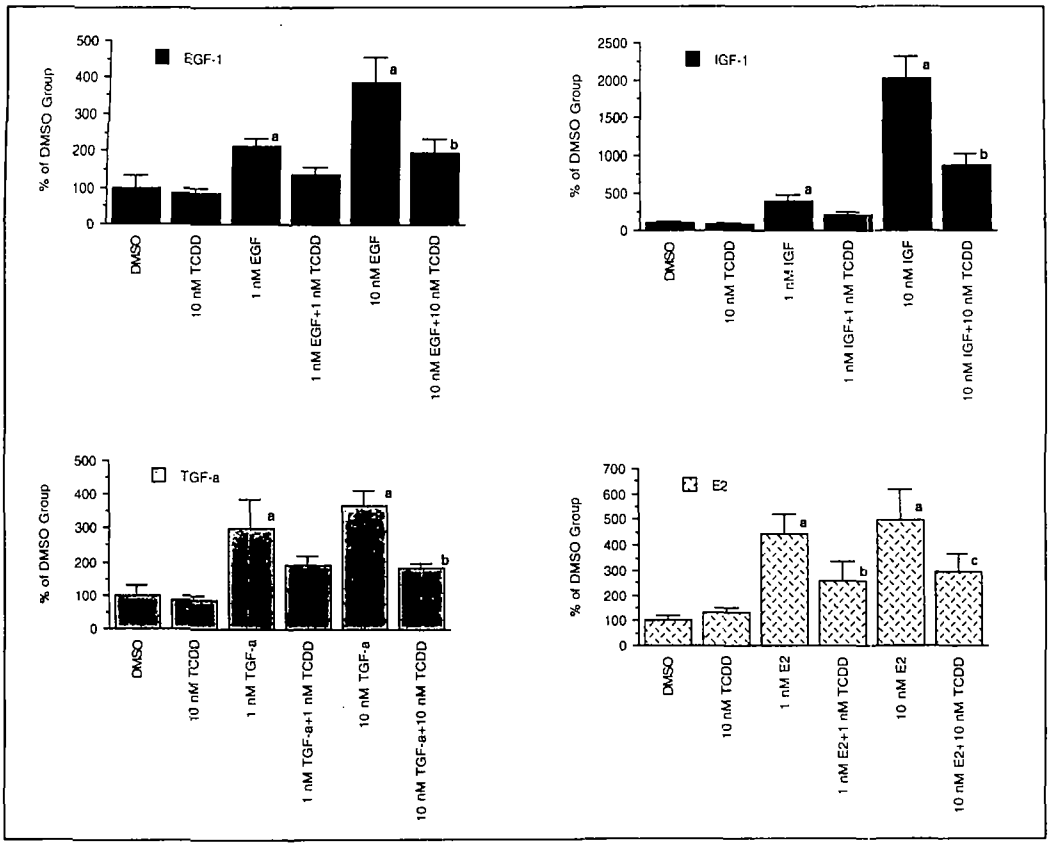

Figure 1. Effects of EGF, IGF-1, TGF and E2 on proliferation of MCF-7 cells and the growth inhibitory activity of TCDD.

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