Inhibition of Estrogen-induced Retinoic Acid Receptor α1 Gene Expression by 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) in MCF-7 Human Breast Cancer Cells

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Introduction

The vitamin A-derived retinoids are a group of compounds that regulate cellular growth and differentiation. The actions of retinoids appear to be mediated by nuclear receptors that belong to the steroid/thyroid hormone receptor superfamily (1-4). Two related classes of retinoid receptors, each consisting of α, β and γ subtype, have been identified: the retinoic acid receptor (RARs) and retinoid X receptor (RXRs). Each type of receptor is known to have multiple isoforms. The function of the different isoforms has not been completely delineated.

Research in several laboratories have demonstrated that RARα plays a role in the growth inhibitory effects of retinoids. It has been reported that ER-positive human breast cancer cells and tumor biopsies exhibit higher levels of RARα mRNA than their ER-negative counterparts (5,6). Retinoids selectively inhibit proliferation of ER-positive human breast cancer cells (6-8). Moreover, transfection of hER or RARα expression vectors into ER-negative or RARα-negative retinoid-resistant breast cancer cells can restore sensitivity to growth inhibition of retinoids. Recently studies have shown that only RARα 1 isoform is induced by E2 in MCF-7 and T47D human breast cancer cells (10-12).

Several studies have been reported that the environmental contaminant TCDD and related compound inhibit both E2- and growth factor-induced proliferation of ER-positive human breast cancer cell lines (13). TCDD also modulated expression of several E2-induced genes including cathepsin D, procathepsin D, the progesterone receptor

(PR), pS2 and epidermal growth factor receptor. Studies on interactive effects of TCDD with retinoic acid have shown both TCDD and RA elicit a number of common responses in MCF-7 human breast cancer cells. Some responses to cotreatment with TCDD plus RA were essentially additive (14), including: inhibition of E2-induced cell proliferation and [^{3}H] thymidine uptake; inhibition of nuclear ER ligand binding and binding of nuclear extracts to a [^{32}P] ERE, and down-regulation of steady-state ER mRNA levels in a time-dependent manner. TCDD also decreases steady-state RARα mRNA levels in MCF-7 cells. This study reports the effects of TCDD on E2-induced RARα gene expression.

Materials and Methods

Cell Culture Maintence and Grouth:

MCF-7 human breast cancer cells were obtained from ATCC and maintained in MEM medium with 10% fetal bovine serum plus 10 ml antibiotic/antimycotic solution and 10 mg insulin.

Transient Transfection Assay:

The construct pRARA12 spaning a region of RARα1 promoter (-509 to +105) linked to a bacterial chloramphenicol acetyl transferase (CAT) reporter gene in pBLCAT2, which was kindly provided by Dr. Pierre Chambon (INSERM, France). Cultured MCF-7 cells were cotransfected with 10 and 1 μg of the pRARA12 and hER constructs respectively, using the calcium phosphate method. Cells were dosed with the appropriate compounds for 48 h and assayed for CAT activity as previously described (16).

Northern Analysis:

The plasmid pSG5 carring 1.9kb full-lengh coding region of the hRARα (15) was kindly provided by Dr. Pierre Chambon (INSERM, France). Total RNA was isolated from the treated cells using RNA STAT 60 extraction procedure. For the RNA analysis, 20 μg of total RNA was electrophoresed on a denaturing agarose gel (1.2%), and transferred to a nylon membrane. The membrane was hybridized with [^{32}P]-labeled cDNA probe. mRNA bands were visualized by autoradiography and quantitated on a Betagen Betascope 603 blot analyzer imaging system. The membrane was stripped and reprobed. RARα mRNA levels were standardized aganist β-tubulin mRNA levels.

Results and Discussion

1. In MCF-7 cells transiently transfected with pRARA12/ hER plasmids, 10^{-9} M E2 caused a 2.5-fold induction of CAT activity and 10^{-8} M TCDD inhibited this

response. α-Naphthoflavone (NF, 10^{-6} M), an Ah receptor antaganist inhibited the antiestrogenic activity of TCDD (Fig. 1).

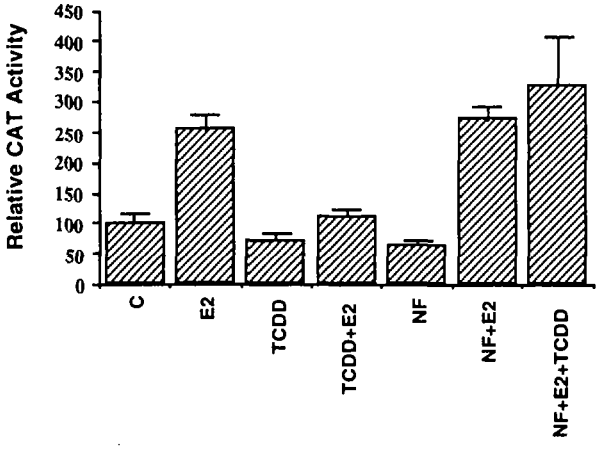

Fig.1. Effects of TCDD and α-Naphthoflavone on Estrogen-Induced CAT Activity in MCF-7 Cells Transiently Transfected with pRARA12 and hER Plasmids

2. In contrast, TCDD did not exhibit antiestrogenic activity in Ah-nonresponsive benzo[a]pyrene-resistant MCF-7 cells (Fig. 2) (16). These data indicate that the antiestrogenic activity of TCDD is mediated through a functional Ah receptor.

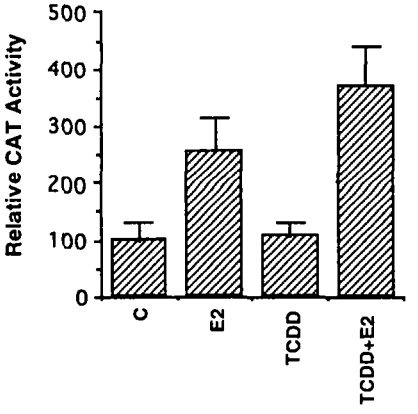

Fig.2. Effects of TCDD on Estrogen-Induced CAT Activity in Benzo[a]pyrene-resistant MCF-7 Cells Transiently Transfected with pRARA12 and hER Plasmids

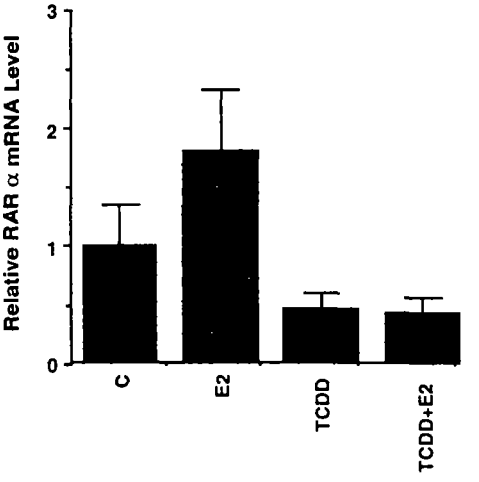

Fig.3. Effects of TCDD on E2-Induced RAR α mRNA Levels in MCF-7 cells

3. The results illustrated in Fig. 3 show that TCDD inhibits E2-induced RARα mRNA levels in MCF-7 cells.

4. These data further extend the antiestrogenic activity of TCDD in MCF-7 cells and current studies are focused on identification of cis-genomic sequences within the RARα1 gene promoter which are required for Ah-responsiveness.

Acknowledgements

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