Effects of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) on the Secretion of TGFβ1 in MCF-7 Human Breast Cancer Cells

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ABSTRACT

Transforming growth factor-β1 (TGFβ1) and TCDD inhibit the growth of MCF-7 breast cancer cells in the presence or absence of 1 or 10 nM 17β-estradiol. MCF-7 cells were treated with DMSO (control), 1.0 and 10 nM TCDD, 17β-estradiol and 1 μM tamoxifen for a period of 7 days and the secretion of TGFβ1 was monitored using commercially available antibodies. Tamoxifen significantly increased the secretion of immunoreactive TGFβ1 whereas no significant effects were observed for the other treatment groups. These data suggest that the growth-inhibitory properties of TCDD are not due to the induced secretion of TGFβ1.

INTRODUCTION

The transforming growth factor β (TGFβ) family of polypeptide hormones stimulate a diverse spectrum of cell-specific intracellular signals and responses. [1] For example, TGFβ stimulates the growth of fibroblasts and can induce anchorage-independent growth of diverse fibroblastic cell lines. In contrast, TGFβ inhibits the growth of various epithelial-derived cells. Several groups have investigated the effects of TGFβ on the proliferation of human breast cancer cell lines and the potential autocrine and paracrine activity of this hormone as a negative growth modulator of these cell lines. [2-6] In one study [4], it was reported that TGFβ inhibited the growth of several estrogen receptor (ER) negative cell lines and these cells contained the TGFβ receptor and secreted the polypeptide into the growth media. In parallel experiments TGFβ did not inhibit the proliferation of several ER-positive cell lines. In contrast, Knabbe and coworkers [2,3] report that TGFβ is secreted by MCF-7 cells and inhibits the growth of both ER-positive and -negative cell lines. Moreover, antiestrogens such as LY 117018, tamoxifen and 4-hydroxytamoxifen, stimulated and 17β-estradiol inhibited the secretion of TGFβ in MCF-7 cells. This study reports the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a potent inhibitor of MCF-7 cell proliferation [7], on the secretion of TGFβ using an antibody

assay procedure.

METHODS

Cell Growth Conditions and Treatment. Porcine TGFβ1 was reconstituted as recommended by the manufacturer (R&D Systems) and diluted with PBS. TCDD and 17β-estradiol (E_2) were dissolved in DMSO and the final concentration of DMSO in media was 0.1%. MCF-7 cells were obtained from ATCC and maintained in MEM with 5% FBS, 6 μg/l bovine insulin and 1 mM sodium pyruvate. For monolayer cell growth assay, the cells were seeded into 35-mm wells in 2 ml ($5x10^4$ cells/well) of MEM/F12 (phenol red free) media supplemented with 3% charcoal-treated CPSR (C-CPSR) and 3% dextran-coated charcoal treated calf serum (DCC-CS) and allowed to attach for 12-18 hours. The media was then changed to 3% C-CPSR, 1% DCC-CS MEM/F12; after 24 hours, fresh media and chemicals were added and the media/chemicals were changed every 48 hours. After 7 days, the cells were trypsinized and the cell numbers were determined using a Coulter counter.

Cell Growth in Conditioned Media and Immunoassay for Secreted TGFβ1. Serum-free conditioned media was prepared as previously described. [8] Subconfluent cells were trypsinized and seeded in 35-mm wells at $2x10^5$ cells/well in 2 ml of MEM/F12 with 3% C-CPSR and 5% DCC-CS. after incubation for 12-16 hours, the wells were washed three times with PBS-0.1% BSA and then changed to serum-free medium (SFM: MEM/F12 with 10 μg/ml transferrin and 200 μg/ml BSA). After 24 hours, the media was replace with fresh SFM with either 0.1% DMSO, 1 nM or 10 nM TCDD, 10 nM 17β-estradiol (E_2), or 1 μM tamoxifen. The media was then changed every 48 hours until the experiment was terminated. The media was collected in siliconized microfuge tubes, clarified by centrifugation at 12,000 *g* for 15 minutes and stored at -70°C after addition of 1 μg/ml each of leupeptin and aprotinin. The cells were trypsinzed and counted. Conditioned media was precipitated with 6% trichloroacetic acid (TCA) for 30 minutes, pelleted by centrifugation at 12,000 *g* for 15 minutes at 4°C and further microfuged for 1 minute. Redissolved pellets were used for quantitation of secreted TGFβ in CM using a sandwich enzyme-linked immunosorbent assay (ELISA) as previously reported [9] with some modifications. Microtiter plates were coated with specific monoclonal mouse antibodies to TGFβ (G enzyme). TGFβ in CM or standard solution was then allowed to bind to these captured antibodies and the resultant immobilized TGFβ was detected by polyclonal rabbit anti-TGFβ antibodies (R&D System) which were detected colorimetrically by the binding of alkaline phosphatase conjugated goat anti-rabbit IgG (Sigma) and incubation with *p*-nitrophenylphosphate (*p*-NPP).

RESULTS

In preliminary studies, treatment of cells with 0, 0.5, 5 and 50 pM TGFβ1 caused a concentration-dependent decrease in the proliferation of MCF-7 breast

cancer cells grown in dextran-coated charcoal stripped media in the presence of absence of 10 nM 17β-estradiol. Figure 1 summarizes the time-course effects of 20 pM TGFβ on the proliferation of MCF-7 cells and demonstrates that there was complete inhibition of cell growth from days 3-7. The results in Figure 2 illustrate the time-dependent secretion of immunoreactive TGFβ1 in MCF-7 cells treated with DMSO (control), 1.0 and 10 nM TCDD, 10 mM 17β-estradiol and 1 μM tamoxifen. The results showed a significant increase in TGFβ1 secretion by cells treated with tamoxifen whereas only a minimum effects on the secretion of TGFβ1 were observed in the other treatment groups.

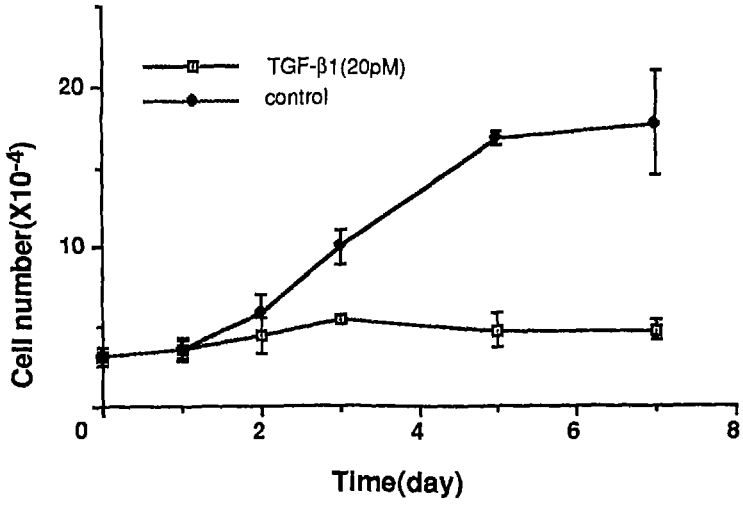

Figure 1. Inhibition of MCF-7 cell growth by 20 pM TGFβ1.

DISCUSSION

TCDD inhibits the proliferation of MCF-7 human breast cancer cells in the presence or absence of 17β-estradiol and exhibits a broad spectrum of antiestrogenic responses in this cell line. [10] In addition, TCDD also downregulates the nuclear ER in this cell line and studies with transcriptional and translation inhibitors suggest that the antiestrogenic effects of TCDD are due to the modulation of gene expression. One possible explanation for the antiproliferative activity of TCDD in MCF-7 cells may be the induced secretion of a potential autocrine growth-inhibitory factor such as TGFβ1. The results in Figure 1 confirm that TGFβ1 inhibits the growth of MCF-7 cells and the antiestrogen tamoxifen, stimulates the secretion of immunoreactive TGFβ1 (Figure 2). These data are consistent with the results of previous studies. [2-6] However, it is also apparent from this study that treatment of MCF-7 cells with

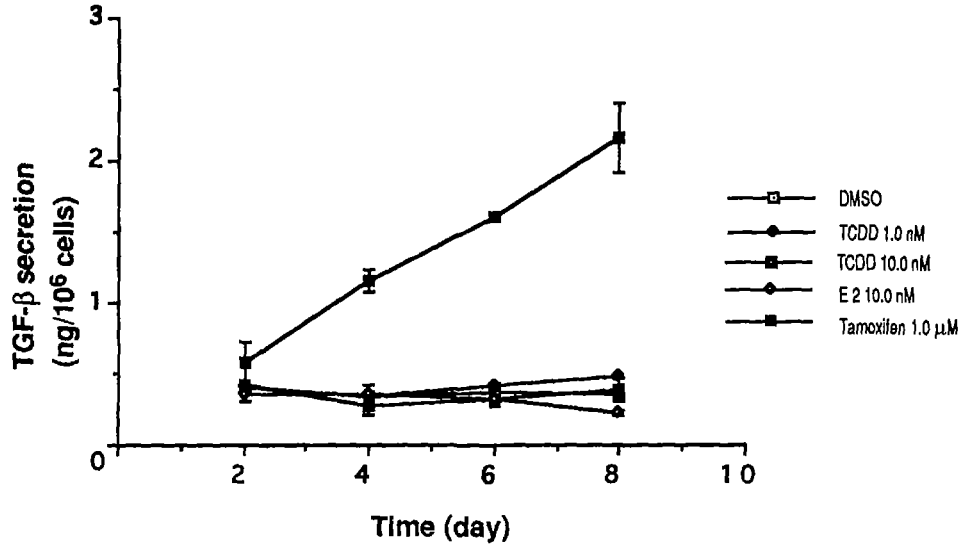

Figure 2. Effects of various treatments on the secretion of immunoreactive TGFβ1.

TCDD does not result in the increased secretion of TGFβ1 (Figure 2) and it is therefore unlikely that the antiestrogenic effects of TCDD in human breast cancer cell lines are due to modulation of cellular levels and the secretion of TGFβ1. (Supported by the National Institutes of Health ES04176).

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