Inhibition of E2-induced *c-fos* Protooncogene Expression by 2,3,7,8-Tetrachlorodibenzo-ρ-dioxin (TCDD) in MCF-7 Human Breast Cancer Cells

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Introduction

The aryl hydrocarbon (Ah) receptor is a ligand-induced nuclear transcription factor that has been widely identified in mammalian tissues, and binds with high affinity to 2,3,7,8-tetrachlorodibenzo-ρ-dioxin (TCDD). TCDD and related compounds elicit a diverse spectrum of tissue/cell-specific biochemical and toxic responses; recent studies in this laboratory and others have characterized the effects of TCDD as antiestrogens in both the female rodent uterus and human breast cancer cell lines. For example, in the rat uterus, TCDD inhibited 17β-estradiol (E2)-induced uterine wet weight, peroxidase activity, epidemal growth factor (EGF) receptor binding and mRNA levels, *c-fos* protooncogene mRNA levels and cytosolic and nuclear progesterone receptor (PR) binding. In human breast cancer cell lines, TCDD also inhibited E2-induced cell proliferation and secretion of the 52-, 34- and 160-kDa proteins and tissue plasminogen activator activity and caused a decrease in nuclear estrogen receptor (ER) and PR levels (1).

c-fos protooncogene plays an important role in regulation of normal cell growth and cellular transformation processes. *c-fos* is a prototypical "immediate-early" gene which is rapidly induced in cells/tissues in response to diverse extracellular stimuli including various mitogens and the steroid hormone 17β-estradiol (E2). The *c-fos* protooncogene encodes a nuclear protein which interacts with *c-jun* to form the heterodimeric AP1 transcription factor complex (2,3). This study reports that TCDD inhibited E2-induced *c-fos* protooncogene mRNA levels in MCF-7 human breast cancer cells. In a time course study, significant inhibition was observed within 2 hr after treating the cells with TCDD. The molecular mechanism of this response was further investigated using the pFC2-BLCAT plasmid which contains the -1400 to +41 5'-flanking sequence from the human *c-fos* protooncogene linked to a bacterial chloramphenicol acetyl transferase (CAT) reporter gene. The results show that 10 nM E2 significantly induced CAT activity (8.5-fold) whereas 10 nM TCDD alone had no significant effects on CAT activity. However, in MCF-7 cells treated with 10 nM E2 plus 10 nM TCDD and transiently transfected with the pFC2-BLCAT plasmid, there was a 46% decrease in E2-induced CAT activity. In contrast, TCDD did not inhibit E2-induced CAT activity in aryl hydrocarbon (Ah)-nonresponsive

benzo[a]pyrene-resistant MCF-7 cells. Co-treatment of 100-fold excess α-naphthoflavone (α-NF) to E2 plus TCDD treated MCF-7 cells prevents TCDD inhibitory effect. This suggests that the antiestrogenic activity of TCDD was associated with the Ah receptor complex.

Materials and methods

Chemicals and Cell culture: TCDD was synthesized in this laboratory to >98% purity as determined by gas chromatographic analysis. E2 was purchased from Sigma Chemical Co.(St. Louis, MO). ^{32}P-radiolabeled dCTP (3000 Ci/mmol) was purchased from NEN (Boston, MA). All other chemicals and biochemicals were of the highest purity available from commercial sources. MCF-7 and benzo[a]pyrene-resistant MCF-7 human breast cancer cells were maintained in DME/F12 media with phenol red and supplemented with 5% fetal bovine serum (FBS), plus 10 ml antibiotic/antimycotic solution (Sigma) in an air/carbon dioxide(95:5) atmosphere at 37°C. The same media without phenol red and FBS-free was then used for at least 48 hr prior to addition of E2, TCDD and other chemicals.

Northern blot analysis: The plasmid containing *c-fos* or β-tubulin genes was purchased from ATCC. RNA was extracted from the cells treated with DMSO(control), 17b-estradiol and/or TCDD using the acidic guanidinium thiocyanate extraction procedure followed by separation on a 1.2% agarose gel electrophoresis and transfer to a nylon membrane. The membrane was then exposed to UV light for 5 min to crosslink RNA to the membrane and baked at 80°C for 2 hr. The membrane was prehybridized in a solution containing 0.1% BSA, 0.1% Ficoll, 0.1% polyvinylpyrollidone, 10% dextran sulfate, 1% SDS and 5X SSPE(0.75 M NaCl, 50 mM NaH2PO4, 5 mM EDTA) for 18 to 24 hr at 65°C and hybridized in the same buffer for 24 hr with the ^{32}P-labeled DNA probe (10^6 CPM/ml). The DNA probes were labeled with (α-^{32}P)dCTP using the random primed DNA labeling kit (Boehringer-Mannheim, Indianapolis, IN). The resulting blots were quantitated using a Betagen Betascope 603 blot analyzer and visualized by autoradiography. *c-fos* mRNA levels were standardized against β-tubulin mRNA.

Transient transfection and CAT assay: pFC2-BLCAT plasmid, which was kindly provided by Dr. Alessandro Weisz(Universita di Napoli, Italy) (3), contains the -1400 to +41 5'-flanking sequence from human *c-fos* gene and a bacterial CAT reporter gene. The cultured MCF-7 and benzo[a]pyrene-resistant MCF-7 cells were transiently transfected with the pFC2-BLCAT plasmid and CAT assays were performed as previously described (4). The percent protein conversion into acetylated chloramphenicol was quantitated using the counts/min obtained from the Betagen Betascope 603 blot analyzer. The TLC plates were subjected to autoradiography using Kodak X-Omat film.

Results and discussion

The results in Figure 1 demonstrate that 10 nM TCDD significantly inhibited E2-

induced *c-fos* mRNA levels within 2 hr of treatment and the levels were decreased for the 24 hr duration of the experiment. At the 2 hr time point, TCDD decreased E2-induced mRNA levels by approximately 50%. These data are similar to those previous reported in the female Sprague-Dawley rat uterus and demonstrated that TCDD blocks one of the early E2- and mitogen-induced responses in E2-responsive target cells (5,6).

The results summarized in Figure 2 show that in MCF-7 cells transiently transfected with the pFC2-BLCAT plasmid and treated with 10 nM E2, there was an 8.5-fold induction of CAT activity. Ten nM TCDD alone had no significant effects on CAT activity. However, in MCF-7 cells treated with 10 nM E2 plus 10 nM TCDD there was a 46% decrease in E2-induced CAT activity. In contrast, TCDD did not inhibit E2-induced CAT activity in aryl hydrocarbon (Ah)-nonresponsive benzo[a]pyrene-resistant MCF-7 cells. In wild-type MCF-7 cells, co-treatment with E2 plus TCDD and 1 μM α-NF blocked the antiestrogenic activity of TCDD. This suggests that the antiestrogenic effects of TCDD were associated with the Ah receptor complex. The potential *cis*-acting genomic sites for the inhibitory effects of the nuclear Ah receptor complex may be the core binding sequences of dioxin-responsive elements (DREs) which have been identified within the 5'-region (-1400/+41) of the *c-fos* gene. The importance of these sequences are being investigated using site-directed mutagenesis and transient transfection studies with plasmids containing specific regions from the 5'-flanking sequence of the *c-fos* protooncogene.

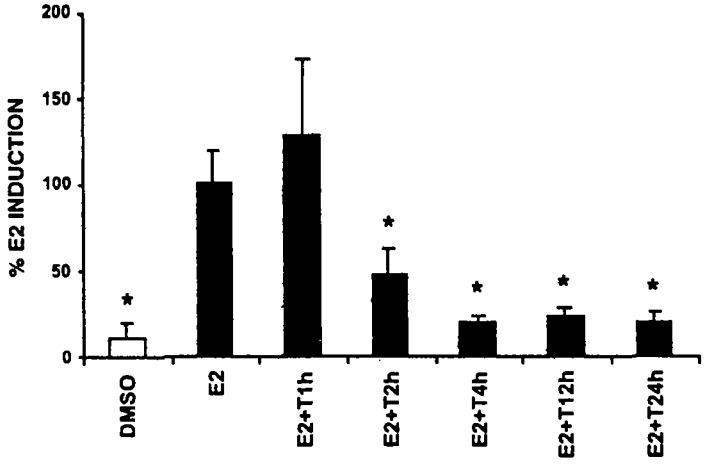

Figure 1. Effects of TCDD on E2-induced *c-fos* mRNA levels in MCF-7 cells. Cells were treated with DMSO (control), 10 nM E2 alone for 1 hr (E2) or co-treated with E2 for 1 hr plus 10 nM TCDD for 1, 2, 4, 12, or 24 hr. *c-fos* mRNA levels were standardized to β-tubulin. * indicates that activity was significantly lower than that of E2 group.

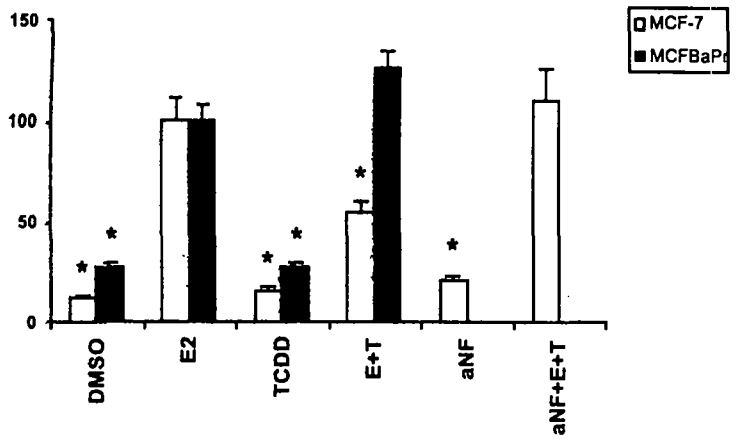

Figure 2. Effects of TCDD on E2-induced CAT activity in MCF-7 or MCF-7^{BaPr} cells transiently transfected with pFC2-BLCAT plasmid. Cells were transiently transfected with pFC2-BLCAT plus plasmid expressing human estrogen receptor (hER) and treated with DMSO (control) , 10 nM E2, 10 nM TCDD, TCDD plus E2 (in both MCF-7 and MCF-7^{BaPr} cells), 1 μM αNF, or αNF plus TCDD and E2 (in MCF-7 cells). CAT activity was determined as described in the Materials and Methods. * indicates activity significantly lower than that of E2 group.

Acknowledgments

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