2,3,7,8-Tetrachlorodibenzo-*p*-dioxin Inhibition of Nuclear AP-1 Binding in MCF-7 Human Breast Cancer Cells

J.C. Rowlands, Xiaohong Wang, S. Safe
Department of Veterinary Physiology & Pharmacology
Texas A&M University, College Station, Tx. 77843-4466

Abstract

Previous studies in this laboratory have demonstrated that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exhibits a broad spectrum of antiestrogenic activities in MCF-7 human breast cancer cells. To further characterize this effect, we present evidence that TCDD can directly inhibit binding by AP-1 to its consensus TRE genomic sequence. AP-1 binding induced by either 12-*O* -tetradecanoylphorbol-13-acetate (TPA) or 17β-estradiol (E2) for 12 hours is blocked within 30 minutes after TCDD treatment. This suggests a direct interaction between AP-1 binding and the signal transduction pathway mediated through the aryl hydrocarbon (Ah) receptor.

Introduction

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related toxic halogenated aryl hydrocarbons elicit a broad range of common toxic and biochemical responses in laboratory animals and mammalian cells in culture.[1-3] Some of these responses include hepatotoxicity and porphyria, immunological changes, carcinogenesis, reproductive toxicity, endocrine effects and induction of a broad range of drug metabolizing enzymes. Among the endocrine effects caused by TCDD is its antiestrogenic activity. For example, TCDD exhibits antiestrogenic activity in rodents by inhibiting estrogen-induced increases in uterine wet weights.[4] TCDD is also inhibits estrogen-induced protein secretion, gene regulation and cell proliferation in the estrogen-responsive MCF-7 human breast cancer cell line.[5,6]

Many factors have been shown to contribute to the proliferation of MCF-7 cells in culture including 17β-estradiol (E2) and peptide growth factors such as insulin and IGF-1.[8] These mitogens initiate signal transduction pathways which can result in the induction of protooncogenes including *c-fos* and *c-myc*. [9] The oncoprotein fos, product of *c-fos*, forms a nuclear heterodimer (AP-1) with members of the jun oncoprotein family. Jun can form homodimers also termed AP-1.[10] AP-1 is a transcription factor which exhibits enhanced affinity for specific cis-acting genomic sequences, namely the 12-*O*-tetradecanoylphorbol-13-acetate (TPA) responsive element (TRE) and thereby stimulates TRE-dependent transcriptional activity.[8] Recently, E2 has been shown to directly

stimulate *c-fos* expression and TRE-dependent activity.[8]This paper will examine the effect of TCDD on TPA and E2-stimulated binding of AP-1 to a TRE, using a gel shift assay procedure.

Materials and Methods

Materials: All chemicals and cell culture media were purchased from Sigma Chemical Company, St. Louis, MO. unless otherwise specified. Fetal calf serum was purchased from Hazelton Biologics, Inc., Lenexa, KS.Plastic cell culture supplies were purchased from Corning Science Products, Corning, N.Y. 2,3,7,8-TCDD was synthesized in this laboratory.

Cell Culture: All cells were grown at 37°C, 95% humidity in an atmosphere of 5% CO2. Stock cells were grown in DMEM-F12 (1:1) Ham medium supplemented with 5% fetal calf serum , 1.2 g/l sodium bicarbonate and 1X antibiotic antimycotic (Ab/Am) solution (100 units penicillin, 100 ug streptomycin and 0.25 ug amphotericin B per ml media).Cells were passed twice a week by lifting the monolayer with trypsin/EDTA. Cells used for nuclear extracts were passed into DMEM-F12 (1:1) Ham medium without phenol red 1.2 g/l sodium bicarbonate, 1X Ab/Am, 0.2% (w/v) BSA, 30 nM NaSeO4, and 10 ug/ml Transferrin (referred to as DF+)[7] supplemented 5% charcoal stripped serum (CSS) for 24 hours after which the cells were washed once with PBS and incubated for 24 hours in DF+ without serum before chemicals (100 ng/ml TPA, 10 nM E2, 10 nM TCDD, 0.1% DMSO) were added.

Nuclear Extract:Pelleted cells were washed twice in 30 ml of HEGD buffer (25 mM HEPES, 1.5 mM EDTA, 1mM dithiothreitol, and 10% glycerol, pH 7.6). The washed cell pellet was resuspended in 3 ml of HED buffer (HEGD buffer without the glycerol) and incubated for 10 minutes on ice. Cells were pelleted and resuspended in 1 ml of HEGD, transferred to a 2 ml homogenizing tube and homogenized using a tight Teflon pestle/drillapparatus. The homogenate was transferred to a centrifuge tube and centrifuged at 4000 x g for 10 minutes. The pellet of nuclei was then resuspended in 100 ul of HEGD buffer containing 0.4 M potassium chloride (pH 7.6) and allowed to stand at 4°C for 1 hour followed by centrifugation at 4°C for 1 hour at 105,000 x g. Nuclei prepared by this method were intact and appeared to be greater than 90% free of extranuclear contamination, as determined by microscopic examination.

Gel Retardation : Complementary strands of the synthetic oligonucleotide containing a consensus TRE sequence was purchased from Promega, Madison Wisconsin. The oligonucleotide was labeled at the 5' end using T4-polynucleotide kinase and [^{32}P]-gATP. Samples of nuclear extract (0.5 mg protein) were incubated in HEDGK (HEGD + 0.4 M KCl) with 200 ng poly (dI-C) for 15 min at 20°C to bind nonspecific DNA-binding proteins. Following the addition of ^{32}P-labeled TRE, the mixture was incubated for a further 15 minutes at 20°C. The reaction mixtures were loaded onto a 5% polyacrylamide gel (acrylamide:bisacrylamide, 30:0.8) and run at 110 V in 90.0 mM Tris borate and 2.0 mM EDTA, pH 8.0. Gels were dried and protein-DNA binding was visualized by autoradiography.

Results and Discussion

Previous studies have demonstrated that the nuclear AP-1 jun-fos heterodimer interacts with the genomic TRE and activates transcription of cell-specific target genes. Several studies have demonstrated that alterations of nuclear AP-1 levels or the jun/fos ratio can markedly influence AP-1 induced responses.[10,11] The effects of TCDD on nuclear AP-1 levels were determined in MCF-7 human breast cancer cells using a gel

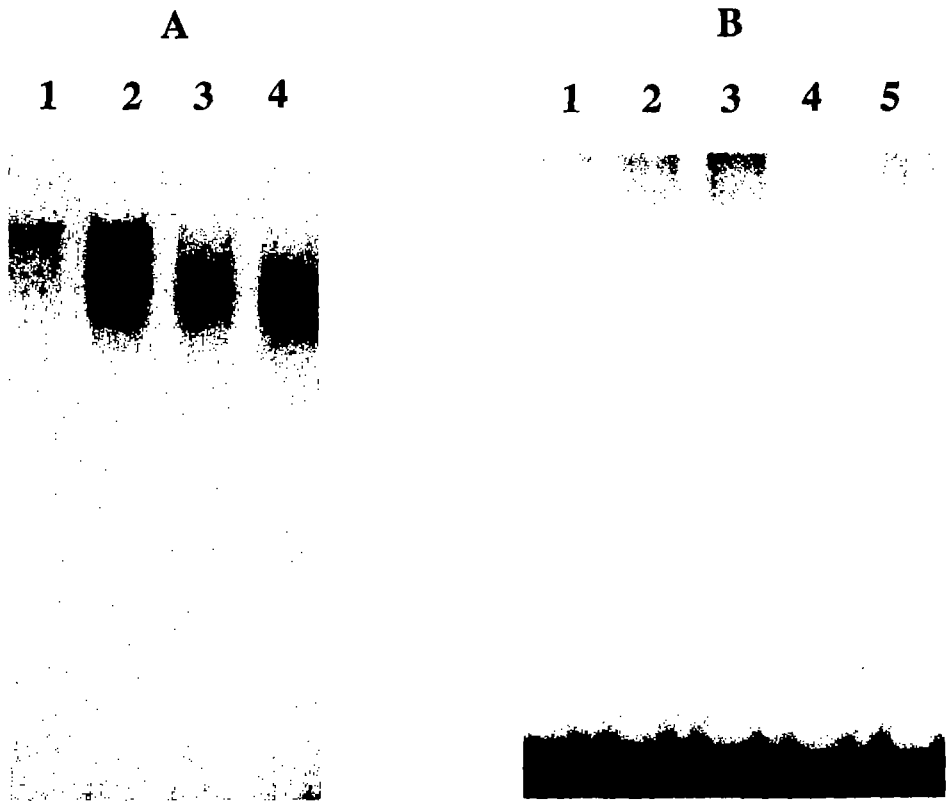

Figure 1. TCDD inhibits AP-1 from binding to a TRE.

(A) Gel shift analysis of nuclear extracts from MCF-7 human breast cancer cells treated with DMSO (lane 1), TPA for 12 hours (lanes 2-4) followed by addition of TCDD for 1.5 hours (lane 3) or 0.5 hours (lane 4) .Nuclear extracts were prepared and incubated with a ^{32}P-labeled TRE (see "Materials and Methods")

(B) Gel shift analysis of nuclear extracts from MCF-7 human breast cancer cells treated with DMSO (lane 1), TCDD (lane 2), E2 for 12 hours (lanes 3-5) followed by addition of TCDD for 1.5 hours (lane 4) or 0.5 hours (lane 5) .Nuclear extracts were prepared and incubated with a ^{32}P-labeled TRE (see "Materials and Methods")

shift assay procedure in which the binding of nuclear AP-1 heterodimers to a synthetic ^{32}P-TRE can be readily detected and quantitated. TPA induces nuclear AP-1 and this is evident in the increased intensity of the retarded band (Figure 1A, lane 2). However, treatment of the cells with TPA plus TCDD caused a time-dependent decrease in the TPA-induced retarded band. To further characterize the influence of TCDD on nuclear AP-1 binding, the MCF-7 cells were also treated with 10 nM E2 which is known to induce *c-fos* proto-oncogene expression. The results illustrated in Figure 1B demonstrate that E2 increases AP-1 binding activity in MCF-7 cells, however in the cotreatment studies, the results indicate that TCDD can block E2 induced AP-1 binding to its target TRE both at 1.5 and 0.5 hours post TCDD treatment.

Recently, several investigators have reported direct interactions between nuclear hormone receptors and members of the AP-1 transcription factor family (reviewed in ref. 11). To our knowledge, this is the first observation that TCDD can directly interfere with the binding of a nuclear transcription factor to its consensus sequence. Our laboratory is currently investigating the mechanism by which this inhibition occurs, and the subsequent effects on the target cells. (Supported by the National Institute of Health, ESO4176)

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