TIME DEPENDENT EFFECTS OF TPA ON TCDD-INDUCED CYP1A1 GENE EXPRESSION IN MCF-7 HUMAN BREAST CANCER CELLS

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ABSTRACT

The effects of 12-O-tetradecanoylphorbol-13-acetate (TPA) on the induction of CYP1A1 gene expression by 2,3,7,8-tetrachlorodibenzo-p -dioxin (TCDD) were investigated in MCF-7 human breast cancer cells. Treatment of cells with TPA prior to TCDD induction had a biphasic effect on CYP1A1 mRNA levels that was dependent upon time of pretreatment with TPA. Pretreatment with TPA for 2 or 6 h resulted in a downregulation of TCDD-induced CYP1A1 mRNA levels, while pretreatment for 12 or 24 h had no effect. However, pretreatment with TPA for 48 or 72 h resulted in superinduction of CYP1A1 by TCDD compared to induction observed with TCDD alone. A similar biphasic effect was also observed for TCDD-induced EROD activity.

INTRODUCTION

The control and regulation of CYP1A1 gene expression has been extensively investigated in both laboratory animals and transformed mammalian cells in culture. The constitutive expression of the gene is low; however, gene expression is induced by aryl hydrocarbon (Ah) receptor ligands such as TCDD, benzo[a]pyrene and 3-methylcholanthrene (MC)[1]. The induced response is a multi-step process which involves the formation of liganded cytocolic Ah receptor which undergoes transformation and nuclear translocation. The nuclear Ah receptor complex acts as a ligand-induced nuclear transcription factor which binds to dioxin responsive elements (DREs) in the 5'-flanking region of induced genes such as CYP1A1[2]. The liganded nuclear Ah receptor complex is a heterodimer which contains the ligand-binding Ah receptor subunit and the aryl hydrocarbon nuclear translocator (Arnt) protein[3]. Several studies have reported that TPA inhibits the induction of CYP1A1 gene expression by Ah receptor agonists in rodents or transformed cell lines. This effect has been attributed to TPA-mediated downregulation of protein kinase C (PKC)[4-6] which is paralleled by decreased formation of transcriptionally active Ah receptor nuclear receptor. The results also suggest that formation of transcriptionally active nuclear Ah receptor complex requires PKC-dependent phosphorylation of both the Ah receptor and the Arnt protein[5,6].

This paper describes the time-dependent effects of TPA on TCDD-induced CYP1A1 gene expression in MCF-7 cells and demonstrates that TPA initially causes a decrease followed by an increase in TCDD-induced CYP1A1 mRNA levels and ethoxyresorufin O-deethylase (EROD) activity.

MATERIALS AND METHODS

Chemicals and Biochemicals TCDD and ethoxyresorufin were synthesized in this laboratory (>98% pure by chromatography and spectroscopic analysis). All other chemicals and biochemicals were purchased from commercial sources. MCF-7 cells were purchased from American Type Culture Collection (Rockville, MD).

Cell Maintenance MCF-7 cells were grown in DME/F12 media supplemented with 5% FBS and 10 ml/L antibiotic-antimicotic solution (Sigma). Cells were treated with TPA (100 ng/ml), TCDD (1 nM), or a combination thereof, using dimethyl sulfoxide (DMSO) (.01% total volume) as the solvent.

EROD Activity Trypsinized cells were plated into 25 cm^2 tissue culture flasks, grown to 70% confluency, and treated for 2, 6, 12, 24, 48 or 72 h, followd by an additional 24 h of 1 nM TCDD plus TPA (100 ng/ml). The cells were harvested by trypsinization and split into two equal portions. One portion was used for measuring CYP1A1 mRNA levels and the second portion of cells was used to measure EROD activity by the method of Pohl and Fouts[7].

CYP1A1 mRNA Levels CYP1A1 mRNA levels were measured using a 1.2 Kb Pst1 fragment of the rat P450c cDNA kindly provided by Dr. Alan Anderson (Laval University, Quebec City, Canada). ß -Tubulin mRNA levels were measured using a 1.3 Kb EcoR1 fragment of mouse ß -tubulin cDNA, a gift from Dr. Masahito Negishi (NIEHS, Research Triangle Park, NC, USA). CYP1A1 mRNA levels were determine as previously described[8].

RESULTS

The results in Table 1 summarize the time-dependent effects of TPA (100 ng/ml) on EROD activity and CYP1A1 mRNA levels in MCF-7 cells treated with 1nM TCDD for 24 h. TCDD alone significantly induced EROD activity and CYP1A1 mRNA levels. Pretreatment of MCF-7 cells for 2 or 6 h with TPA followed by a 24 h incubation with TPA+TCDD resulted in a significant decrease in both EROD activity and CYP1A1 mRNA levels. In cells pretreated for 12 or 24 h with TPA there was no significant change in either TCDD-induced response. However, for cells pretreated for 48 or 72 h with TPA and an additional 24 h with TPA + TCDD there was a 2.8- and 2.2-fold increase in EROD activity and CYP1A1 mRNA levels respectively.

Table 1. Time-dependent effects of TPA on TCDD induced CYP1A1 gene expression in MCF-7 human breast cancer cells.[a]

Treatment	Duration of TPA Pretreatment (h)	EROD Activity (pmol/min/mg)	Relative mRNALevels
TCDD	0	122 ± 12.5	36 ± 3
TCDD + TPA	2	25.5 ± 2.8[b]	20 ± 8[b]
	6	39.0 ± 6.0[b]	15 ± 6[b]
	12	123 ± 14.2	32 ± 7
	24	132 ± 3.6	47 ± 15
	48	315 ± 16.7[c]	74 ± 20[c]
	72	345 ± 60.5[c]	80 ± 20[c]
DMSO	-	ND	13 ± 3

[a] The MCF-7 cells were treated for 24 h with 1 nM TCDD in the presence or absence of 100 ng/ml of TPA. The EROD activity and CYP1A1 mRNA levels were determined and the results are expressed as means ± SD for experiments carried out in triplicate; TCDD induces both EROD activity and CYP1A1 mRNA levels; treatment with TPA significantly ($p<0.01$) [b] decreased or [c] decreased ($p<0.01$) the TCDD induced responses compared to the values in cells treated with TCDD alone.

DISCUSSION

Both TPA and TCDD are tumor promoters which elicit a diverse spectrum of organ/tissue and cell-specific responses through different mechanistic pathways. In MCF-7 cells, TPA and TCDD both cause the downregulation of the nuclear estrogen receptor, inhibition of cell proliferation and a number of estrogenic responses. Initial studies in this laboratory have demonstrated that TPA blocks TCDD-mediated inhibition of estrogen-induced metabolism of glucose to lactate; however, this was not accompanied by any significant modulation of TCDD-induced CYP1A1 gene expression[9]. These data are in contrast with several reports which demonstrate that TPA significantly inhibited CYP1A1 gene expression[4-6]. The differential responsiveness of the MCF-7 cells may have been due to the low concentration of TPA (0.1 ng/ml) used in the study[9] and therefore the interactions of TPA and TCDD was reinvestigated using a higher concentration of TPA (100 ng/ml). The results in Table 1 demonstrate that TPA at this concentration significantly modulated TCDD-induced CYP1A1 gene expression. In agreement with previous reports, this study demonstrates that at early time points TPA inhibits TCDD-induced EROD activity and mRNA levels. However, this study also shows that after prolonged exposure of MCF-7 cells to TPA, TCDD causes superinducibility of CYP1A1 gene expression.

Previous studies have reported that treatment of MCF-7 cells with TPA for 12 h is sufficient to downregulate PKC activity[10,11]. Moreover, even after removal of TPA, the PKC activity remains suppressed for up to 11 days. The superinducibility of the CYP1A1 gene after 72 h of exposure to TPA suggests that other pathways may also play a role in the formation of the transcriptionally active Ah receptor complex and this is currently being investigated in our laboratory (Supported by the National Institutes of Health, ES03843).

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