# ROLE OF TARGET STEROID HORMONES IN CELLULAR RESPONSIVENESS TO CYP1A1 INDUCTOIN BY 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) IN MCF-7, RL95-2 AND LNCAP CELLS

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## Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds elicit a diverse spectrum of biological and toxicological responses. Most if not all, of these effects are thought to be mediated by binding to the aryl hydrocarbon receptor (AhR), a ligand activated transcription factor and a member of the basic helix-loop-helix per-arnt-sim family of proteins[1,2]. The mechanism of action of TCDD is similar to that proposed for the intracellular actions of steroid hormones[3,4]. Ligand binding to AhR facilitates the release of HSP90 and its translocation to the nucleus where liganded AhR heterodimerizes with the aryl hydrocarbon receptor nuclear translocator (ARNT) and binds to specific gene regulatory sequences, called xenobiotic response element (XRE). Upon binding to XREs, the heterodimeric AhR-ARNT complex activates transcription of several genes, encoding especially cytochrome P450s that are involved in xenobiotic compound metabolism. Among the genes induced, *CYP1A1* gene expression is the most sensitive and early biochemical response and is therefore used as a response marker gene for TCDD[5,6]. TCDD modulates various endocrine functions by enhancing lignd metabolism[7], altering hormone synthesis[8], down regulating receptor levels[9], and interfering with gene transcription[10, 11]. Its interference with transcription has been thought to be caused by competition between steroid hormone receptors and the liganded AhR-ARNT complex for XREs on steroid-induced genes [10, 12].

In the present investigation, we examined the responsiveness to steroid hormones of TCDD-induced gene expression in three hormone-dependent cell lines, MCF-7, RL95-2 and LNCaP.

## Materials and Methods

### *Materials*

The expression plasmid for the human ER-α was a kind gift from Dr. P. Chambon (IGBMC, INSERM, France). The $E_2$-responsive reporter plasmid, pGL3-3 (EREc38)-LUC, containing three head-to-tail tandem copies of the consensus estradiol response element (ERE), and the androgen-responsive reporter plasmid (MMTV-LUC) were gifts from Dr. C. M. Klinge (University of Louisville, USA) and Dr. K. Umesono (Institute of Virus Research, Kyoto University), respectively. The TCDD-responsive reporter plasmid, pGL3-1 (XRE)-LUC, was prepared by cloning an oligonucleotide containing single XRE elements into the *Bgl*II site of the pGL3 promoter vector (Promega, USA).

*Cell Culture and Treatments*

MCF-7 (human breast carcinoma), RL95-2 (moderately differentiated human endometrial carcinoma) and LNCaP-FGC (human prostate carcinoma) cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA). MCF-7 and RL95-2 cells were routinely maintained in DMEM/Ham's F12 (1:1) medium and LNCaP cells in RPMI 1640 medium. The media were supplemented with 10% fetal bovine serum (FBS), 100 units penicillin/ml and 100 μg streptomycin/ml, under standard conditions in a 37°C incubator with a humidified mixture of 5% $CO_2$ and 95% air. For TCDD experiments, cells were grown on 60-$mm^2$ tissue culture dishes in routine culture media, and at about 80-90% confluency they were treated with different concentrations of TCDD in 0.1% DMSO (v/v) for different time periods. The controls received 0.1% DMSO.

*Ethoxyresorufin O-deethylase (EROD) Assays*

EROD activities of the crude homogenate were measured spectrofluorometrically as described previously [13]. Protein concentrations were measured with a Coomassie protein assay kit (Bio Rad, USA).

*RT-PCR Analysis*

Total RNA was prepared from cells by using Isogen (Nippon Gene, Japan) according to the manufacturer's instructions and RT-PCR was carried out with a RT-PCR kit (TaKaRa Biomedicals, Japan).

*Transfections and Reporter Assays*

Cells were seeded onto 24-well tissue culture plates and grown in normal growth medium. After 24 h, the cells at 50-60% confluency were transfected with DNA by using LipofectAMINE Plus reagents (Life Technologies, USA) according to the manufacturer's instructions for reporter gene assays. The transfected DNA mixture per well included 200 ng of each reporter plasmid, pGL3-3 (EREc38)-LUC, pGL3-1 (XRE)-LUC or MMTV-LUC, along with four ng of pRL-SV40 control plasmid (Promega, USA). In some experiments, ER-α expression plasmids (50-100 ng/well) were co-transfected with pGL3-1 (XRE)-LUC or MMTV-LUC.

*Statistical Analysis*

Statistical analyses were performed by analysis of variance followed by Student's *t*-test. A value of $p<0.05$ was considered statistically significant.

**Results and Discussion**

Exposure to TCDD induced the *CYP1A1* gene in all three cell lines. MCF-7 and RL95-2 cells showed more than 15- and 10-fold induction of EROD activity, respectively, compared with the less responsive LNCaP cells. The steady-state levels of expression of aryl hydrocarbon receptor (AhR) and aryl hydrocarbon receptor nuclear translocator (ARNT) were similar in all three cell lines. Expression of the *CYP1B1* and *PAI-2* genes was induced by TCDD in MCF-7 and RL95-2, but not in LNCaP, cells. Transient co-expression of estradiol receptor-α (ER-α) with a TCDD-responsive reporter plasmid and subsequent TCDD treatment increased responsiveness to TCDD in RL95-2 and LNCaP cells. The induction of CYP1A1 appears to be related to their cellular ER-α contents [14] and that Ah responsiveness is not only dependent on the expression of AhR but also on ER-α levels [14-16].

Although RL95-2 cells showed an approximately 10-fold increase in EROD activity compared with LNCaP cells, both showed similar levels (3-fold) of induction of TCDD-responsive reporter gene activity driven by a single XRE element. This could not be explained by ER-α content, since ER-α expression was similar, or possibly lower, in LNCaP cells, and thus it suggests the involvement of some other factor(s). Treatment with AZA-C, a DNA methyltransferase inhibitor, enhanced responsiveness to TCDD, in terms of EROD activity in LNCaP cells, but not in MCF-7 and RL95-2 cells, suggesting that DNA methylation in the CpG dinucleotide within the XRE core sequence is another factor involved in silencing of CYP1A1 in LNCaP cells.

TCDD markedly inhibited $E_2$- or testosterone-induced reporter gene activities in all three cell lines. Conversely, these target hormones inhibited TCDD-induced EROD activity in the three cell lines. In LNCaP cells, however, not only testosterone but $E_2$ and progesterone also showed an inhibitory effect. This is probably because LNCaP cells have a mutant AR (Thr877 to Ala in the ligand binding domain) to which androgens, progestagens and estrogens all bind strongly and stimulate cell growth or androgen-regulated reporter gene activity [17, 18].

Inhibitory effects of target steroid hormones in the present study would appear to contradict our own observation of increasing transactivation of TCDD-responsive reporter gene activity by ER-α. We propose that when ER-α is in an unliganded state, it might interact with other transcription factors such as nuclear factor-1[19] and transactivate CYP1A1 induction. However, the liganded homodimeric form of ER-α might be unable to interact with these transcription factors but at the same time might interfere with the binding of the AhR-ARNT complex to its cognate XRE element, as shown previously [10].

In conclusion, this study has demonstrated that target hormones affect 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-inducible gene expression in an experimental model system consisting of three human cancer cell lines. The findings indicate that TCDD and the target steroid hormones negatively regulate each other's activity in the target cells.

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