# INDUCTION OF CYP1A1 AND CYP1B1 BY TCDD IN THE HUMAN BREAST EPITHELIAL CELL LINE: IMPLICATIONS FOR REDOX ACTIVE CATECHOL ESTROGEN FORMATION

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## Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), a prototype agonist of the aromatic hydrocarbon (Ah) receptor, has been shown to induce various forms of cytochrome P450 isoforms responsible for oxidative metabolism of a wide array of endogenous as well as xenobiotic substances. Human cytochrome P450 1A1 (CYP1A1) and 1B1 (CYP1B1) are known to catalyze the hydroxylation of 17-beta-estradiol (E2) at C-2 and C-4 positions, respectively[1]. The resulting 2-hydroxyestradiol (2-$OHE_2$) and 4-hydroxyestradiol (4-$OHE_2$) have been considered to undergo redox cycling to produce reactive oxygen species (ROS) that could cause oxidative DNA and tissue damage associated with hormonal carcinogenesis[2,3]. In the present study, we have examined the induction of CYP1A1 and CYP1B1 isoforms in the cultured human breast epithelial cell line (MCF10A) and the oxidative cell death induced by resulting catechol estrogens.

## Materials and Methods

*Cell culture*: The MCF10A cells were maintained at 37°C in a humidified atmosphere of 5% $CO_2$/95% air in DMEM/F12 medium supplemented with 5% heat-inactivated horse serum, 10 μg/ml insulin, 100 ng/ml Cholera toxin, 0.5 μg/ml hydrocortisone, 20 ng/ml recombinant EGF, 2 mM L-glutamine, 100 ng/ml penicillin/streptomycin/fungi zone mixture. Cells were grown to 60-80% confluence and trypsinized with 0.05% trypsin containing 2 mM EDTA.

*Determination of cell viability and proliferation*: The viability of MCF10A cells was determined by the conventional MTT reduction assay. The cell proliferation was assessed by measuring the incorporation of tritium ($^3H$)-labelled thymidine into nuclei.

*Measurement of intracellular ROS generation*: To monitor net intracellular accumulation of ROS, the fluorescent probe dichlorofluorescein diacetate (DCF-DA) was used. Cells were rinsed with Kreb's ringer solution and 10 μM DCF-DA was loaded. After 10 min incubation at 37°C, cells were examined under a confocal microscope equipped with an argon laser.

*Western blot analysis of CYP1A1 and CYP1B1*: Protein levels of CYP1A1 and CYP1B1 were determined by immunoblot analysis using the corresponding antibodies. Their mRNA transcripts were analyzed by using [□-$P^{32}$]-labelled cDNA probes.

*Measurement of poly(ADP-ribose)polymerase (PARP) cleavage*: Cleavage of 115 Kd PARP to the 85 Kd fragment was assessed using mouse anti-PARP antibody (BIOMOL Res. Lab., Plymouth, PA, USA).

**Results and Discussion**

Treatment of MCF10A cells with TCDD led to time- and concentration-related induction of both CYP1A1 and CYP1B1 proteins (Fig. 1). Resveratrol, a chemopreventive phytoalexin present in grapes and red wine, strongly inhibited TCDD-induced CYP1A1 and CYP1B1 expression at both transcriptional and translational levels (Fig. 2 and Fig. 3). Induction of CYP1A1 and CYP1B1 is associated with hydroxylation of 2-$OHE_2$ and 4-$OHE_2$, respectively. When MCF10A cells were exposed to each catechol, there was concentration-dependent decrease in the cell viability and proliferation (data not shown). Both 2-$OHE_2$ and 4-$OHE_2$ caused cleavage of PARP (Fig. 4) and positive *in situ* terminal end-labeling (TUNEL) in MCF10A cells, indicative of occurrence of apoptotic cell death. The cytotoxicity of 2-$OHE_2$ was ameliorated by the antioxidant *N*-acetyl-L-cysteine, suggesting that this catechol estrogen causes oxidative cell death through generation of ROS. In support of this assumption, MCF10A cells treated with 2-$OHE_2$ exhibited enhanced accumulation of ROS (Fig. 5) as revealed by increased fluorescence derived from intracellular peroxide reacting with DCF dye. As illustrated in Fig. 6, 2-$OHE_2$ treatment also resulted in the activation of c-Jun-$NH_2$ protein kinase (JNK), an important mitogen-activated protein kinase involved in mediating apoptotic signals. The activation of JNK via phosphorylation is frequently observed in cells undergoing apoptotic death[4,5]. In conclusion, treatment of MCF10A cells with TCDD markedly induced expression of both CYP1A1 and CYP1B1 and their mRNA transcripts responsible for the formation of 2-$OHE_2$ and 4-$OHE_2$, respectively. The resulting catechol estrogens caused oxidative cell death via apoptosis, as assessed by PARP cleavage and positive TUNEL staining. Resveratrol significantly inhibited induction of both CYP1A1 and CYP1B1 in MCF10A cells stimulated with TCDD, which may provide another line of molecular mechanisms underlying the chemopreventive activity of this phytochemical.

**Acknowledgements**

This work was supported by the NITR/Korea FDA Grant ED2000-26 for Endocrine Disrupter Research.

**References**

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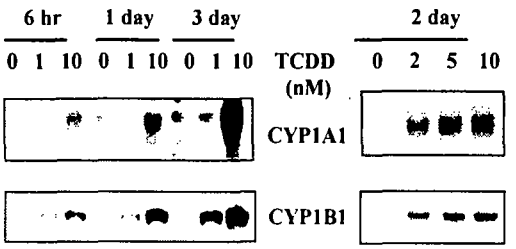


Fig. 1. TCDD-induced increases in CYP1A1 and CYP1B1 expression in MCF10A cells.

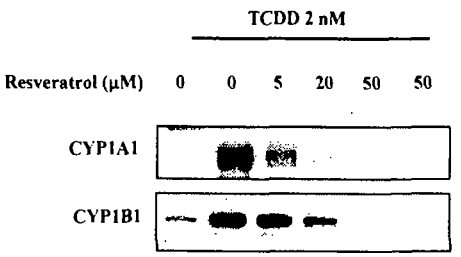


Fig. 2. Effects of resveratrol on TCDD-stimulated expression of CYP1A1 and CYP1B1 in MCF10A cells.

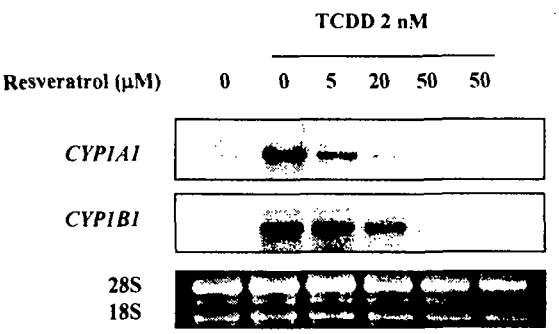


Fig. 3. Effects of resveratrol on TCDD-stimulated *CYP1A1* and *CYP1B1* mRNA expression in MCF10A cells.

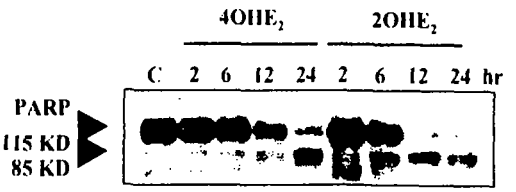


Fig. 4. Cleavage of poly (ADP-ribose) polymerase (PARP) by catechol estradiols in MCF10A cells.

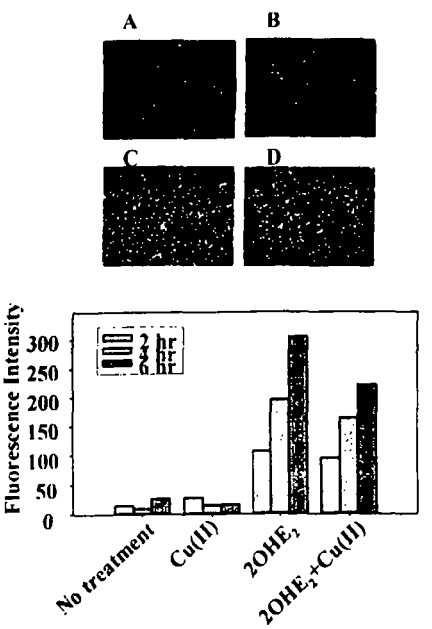


Fig. 5. 2-Hydroxyestradiol-induced intracellular accumulation of ROS.

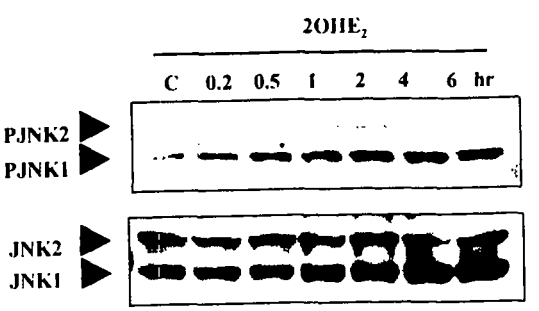


Fig. 6. Kinetics of 2-hydroxyestradiol ($2OHE_2$)-induced JNK Activation in MCF10A cells.