DOWN-REGULATION OF TCDD-INDUCIBLE CYP1A1 EXPRESSION BY o,p'-DDT

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Introduction

Dichlorodiphenyltrichloroethane (DDT) is an organochlorine pesticide still widely used in developing countries for the control of malaria and other vector-transmitted diseases. The chemical characteristics of DDT compounds favor its accumulation and bioconcentration in lipid systems of all species, leading to continuous exposure and potential adverse effects to humans and wild animals. Although the more evident toxic signs during acute exposure to DDT occur in the central nervous system, some studies have suggested that chronic exposure alters sexual steroidal hormone homeostasis in wild and experimental animals [1-3]. These findings have led to DDT and its metabolites being considered as endocrine disruptors able to promote hormone dependent pathology [4].

Several studies have shown that DDT alters the activity of many microsomal enzyme activities, including those involved in phase I and phase II metabolism of xenobiotics [5,6]. There have shown that DDT compounds induce hepatic CYP-dependent microsomal monooxygenases in different species [7-10]. The effects, determined either by immunoreactive proteins or catalytic activities, consisted mainly of a preferential induction of CYP2B subfamily, a lesser induction on CYP3A, and minimal or no induction of CYPIA. On this basis, DDT has been considered a PB type of inducer [6,9,10]. Although there has been an active investigation on the inductive capacity of DDT compounds on hepatic P-450s, the molecular basis for the regulation of P450s by o,p'-DDT has not been well elucidated. o,p'-DDT has been reported to have estrogenic activity [4]. We previously reported that estradiol suppressed CYP1A1 in Hepa-1c1c7 cells [11]. However, the effects of o,p'-DDT on the regulation of CYP1A1 have not been described. In the present study, we investigated the effect of o,p'-DDT on TCDD-inducible CYP1A1 gene expression in mouse hepatoma Hepa-1c1c7 cells.

Methods and Materials

Materials: Chemicals and cell culture materials were obtained from the following sources: o,p'-DDT (>99% pure: Alderich); 7-ethoxyresorufin and resorufin (Pierce Chemical Co.); TCDD (Chemsyn Science Lab.); LipofectAMINE Plus, αMEM, fetal bovine serum, penicillin-streptomycin solution, and trypsin (Life Technologies, Inc.); pCMV-β-gal (Clonetech).

Cell culture and treatment: The mouse hepatoma Hepa-1c1c7 cells were cultured in a α-MEM supplemented with 10% fetal bovine serum at 37°C in a humidified 5% CO_2 incubator. o,p'-DDT and TCDD were dissolved in dimethylsulfoxide. Stock solutions of these chemicals were directly added to the culture media and incubated with o,p'-DDT or/and TCDD for 18 h. Control cells were treated only with solvents, the final concentration of which never exceeded 0.2%.

7-Ethoxyresorufin O-deethylase (EROD) assay: Hepa-1c1c7 cells were incubated with 0.5 nM

TCDD in the presence of dimethylsulfoxide (vehicle control) or o,p'-DDT for 18 h. EROD activity, assayed as the bioactivation capacity of CYP1A1, was determined as described previously [12].

RNA preparation and CYP1A1 mRNA analysis by RT-PCR. Hepa-1c1c7 cells were incubated with 0.5 nM TCDD or/and bisphenol A for 6 h. Total cellular RNA was isolated by the acidic phenol extraction procedure. cDNA synthesis, semiquantitative RT-PCR for CYP1A1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA, and analysis of results were performed as described previously [13].

Transfection and Luciferase and β-Galactosidase Assays: Hepa-1c1c7 cells (2.5×10^5) were transiently co-transfected with the plasmids pCyp1a1-Luc and pCMV-β-gal using LipofectAMINE Plus. Four hours after transfection, cells were treated with vehicle, TCDD, or o,p'-DDT. Following exposure for 18 h, luciferase and β-galactosidase activities were determined. Luciferase activity was normalized using β-galactosidase activity and was expressed as relative to the activity detected with the vehicle controls.

Statistical Analysis: All experiments were repeated at least three times. Student's t-test was used to assess the statistical significance of differences. A confidence level of < 0.01 was considered significant.

Results and Discussion

We investigated the effects of o,p'-DDT on Ah (aryl hydrocarbon) receptor-mediated induction of the CYP1A1 gene expression in Hepa-1c1c7 cells. To this end, we used TCDD, a prototypical inducer of the CYP1A1 that binds to the Ah receptor with a high affinity, on induction of the CYP1A1 gene expression. EROD activity is considered to be a convenient measure of the activity corresponding to the CYP1A1 gene [11,14]. Following treatment of cells with TCDD, there was markedly increased in EROD activity compared with the control (Fig. 1). o,p'-DDT alone did not affect EROD activities. However, TCDD-inducible EROD activities were significantly reduced in cultures co-treated with o,p'-DDT and TCDD with a dose-dependent manner (Fig. 1). The o,p'-DDT -mediated suppression of EROD induction was not due to a cytotoxic effect of the o,p'-DDT. The total number of cells per culture dish and the viabilities of attached cells were identical for cultures treated with bisphenol A (data not shown).

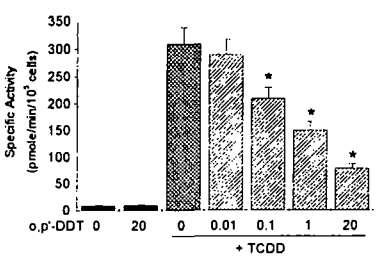

Fig. 1. Effects of o,p'-DDT on EROD activity in Hepa-1c1c7 cells. EROD activities were measured in Hepa-1c1c7 cells that had been treated with TCDD (0.5 nM) or/and various concentrations o,p'-DDT (0.01 ~ 20 μM) for 18 h as described in Materials and Methods. Values present as the mean ± SD triplicate cultures. *Significantly different from TCDD.

The effects of o,p'-DDT on TCDD-induced induction of CYP1A1 mRNA level was determined by RT-PCR. Consistent with the results obtained from the EROD activity assay, CYP1A1 mRNA levels were markedly suppressed by co-treatment with o,p'-DDT and TCDD (Fig. 2). Therefore, suppression of CYP1A1 by o,p'-DDT is speculated to be regulated through transcriptional activation and o,p'-DDT could inhibit Ah receptor-mediated gene expression.

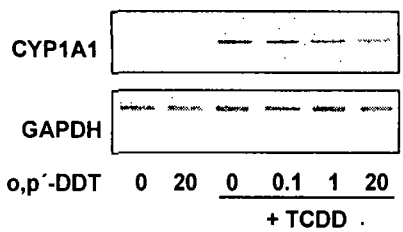

Fig. 2. RT-PCR analysis of CYP1A1 mRNA in Hepa-1c1c7 cells. Hepa-1c1c7 cells were treated with o,p'-DDT (0.1 ~ 20 μM) or/and TCDD (0.5 nM) for 6 h. Total cellular RNA was isolated from cells. For CYP1A1 and GAPDH expression, their cDNAs, which were reverse transcribed from total RNA, were amplified by PCR.

The control and regulation of the CYP1A1 gene expression have been extensively investigated. It is currently believed that the CYP1A1 gene expression is induced by the TCDD and related Ah receptor agonist through the Ah receptor-mediated signal transduction. Binding of TCDD to the Ah receptor results in transformation of the receptor into a DNA binding protein. The ligand-receptor complex recognizes DRE located in the 5'-regulating region of the CYP1A1 gene and promotes induction of transcription [15]. To assess whether this inhibition was transformed the Ah receptor by o,p'-DDT to a form that dose not recognize the DRE in the 5'-regulating region of the CYP1A1 gene hence decreased the level of CYP1A1 transcription, Hepa-1c1c7 cells were transiently transfected with pCyp1a1-Luc reconstructed reporter plasmid which contains a sequence of the CYP1A1 gene (-1306 to –824) that encompasses four copies of the DRE enhancer sequence located upstream of a structural gene coding for the luciferase. Cells were treated with TCDD and/or o,p'-DDT and luciferase activities were determined. TCDD treatment resulted in an increase in luciferase activity compared with control. However, when the cells were treated simultaneously with both TCDD and o,p'-DDT, the luciferase activity was significantly reduced relative to the cells treated with TCDD alone (Fig. 3).

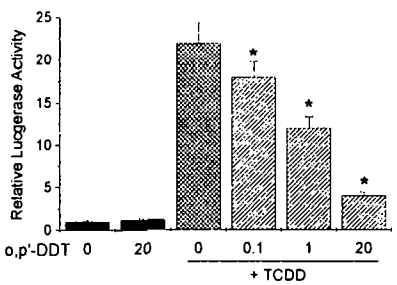

Fig. 3. Effects of o,p'-DDT on luciferase activity in Hepa-1c1c7 cells transiently transfected with pCyp1a1-Luc. Hepa-1c1c7 cells were transiently transfected with pCyp1a1-Luc, which contains the 5'-regulating region of the CYP1A1gene, and then treated with TCDD (0.5 nM) or/and o,p'-DDT (0.1 ~ 20 μM) for 18 h. Cells were harvested, and luciferase activities were. Values ± SD, each performed in triplicate. The enzyme activities were expressed as relative to that seen with the vehicle (VH) alone. *Significantly different from TCDD.

This result indicates that the actions of o,p'-DDT on inhibiting TCDD-induced CYP1A1 gene expression is a result in decrease of Ah receptor-dependent transcriptional activation by alternation in the DRE binding potential of nuclear Ah receptor or a block in transport to the nucleus based upon the information pertaining to the physical and hydrodynamic properties of the Ah receptor. However, we could not exclude other possibilities that o,p'-DDT may exert indirect transcriptional interference between other transcription factors binding to negative regulatory element region in *CYP1A1 promoter* region or changes in the phosphorylation state of the cytosolic receptor complex. It is also possible that the inhibitory effect of o,p'-DDT on TCDD-inducible CYP1A1 gene expression may be a result of one or more of its metabolites irreversibly inactivating an essential component of the CYP1A1 gene expression system.

The estrogen receptor and Ah receptor are co-expressed in several Ah and estrogen-responsive cell lines. Some studies indicated that the estrogen receptor is important for Ah receptor-mediated transactivation in breast cancer cells, such as MCF-7 cells [16]. A study reported that estradiol inhibited Ah responsiveness in mouse Hepa-1c1c7 cells [17], and therefore, demonstrated the presence of a two-way cross-talk between the intracellular signaling pathway involving estrogen and Ah. Additional studies including the involvement of estrogen receptor in down-regulations of TCDD-inducible CYP1A1 expression by o,p'-DDT and further studies to elucidate the mechanism are in progress.

Acknowledgments
This work was supported by a endocrine disruptor grant from the Korea Food & Drug Administration, Republic of Korea.

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