# INDUCTION OF CYP1A1 GENE TRANSCRIPTION BY TCDD IN HUMAN OVARIAN CANCER CELLS.

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## ABSTRACT

The three human ovarian cancer cell line, PEO-1, PEO-4, and PEO-6, express both the estrogen receptor and the aryl hydrocarbon (Ah) receptor which accumulates in the nucleus of these cells. Treatment of these cells with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) significantly induced CYP1A1 mRNA or the associated monooxogenases - aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-O-deethylase (EROD) activities in only the PEO-4 cell line. In gel shift assays, only the nuclear Ah receptor complex from TCDD-treated PEO-4 cells, bound to the dioxin-responsive element (DRE). In this study, ovarian cancer cells were pretreated with cycloheximide ($10^{-5}$ M; 1 hr), then co-treated with TCDD for 5 hr. The TCDD-induced CYP1A1 mRNA levels in PEO-4 cells were superinduced in cells pre-treated with cycloheximide. CYP1A1 mRNA levels were also induced in PEO-6 cells co-treated with TCDD plus cycloheximide, whereas CYP1A1 mRNA levels in PEO-1 cells were only marginally increased. The results suggest that the lack of TCDD-induced CYP1A1 gene expression in PEO-6 cells may be due in part to negative regulation by protein factor(s) which are inhibited by the cycloheximide treatment.

## INTRODUCTION

The mechanism by which TCDD induces gene expression is not yet completely understood . We chose to study the activity of TCDD in three human ovarian cancer cell lines: PEO-1, PEO-4, and PEO-6 cells. Sucrose density gradient profiles have shown that all three cell lines contain cytosolic Ah receptor, which undergoes nuclear translocation after the cells have been treated with TCDD. However, TCDD responsiveness, as measured by the induction of aryl hydrocarbon hydroxylase (AHH) activity, was only significant in the PEO-4 cells (*Rowlands, et al*, 1993).

Some of the possible reasons for the lack of AHH induction in these Ah-receptor positive cell lines have been investigated in this study. Despite the expression of the Ah receptor in all 3 cell lines, and the TCDD-induced formation of a DNA (dioxin responsive element, DRE) binding nuclear complex, CYP1A1 gene expression was induced by TCDD, only in PEO-4 cells. The identification of a putative negative regulatory element (NRE) in the 5' flanking region of the CYP1A1 gene and the observation that CYP1A1 message is superinduced by treatment with TCDD after pre-treatment with cycloheximide led us to test the possibility that a transiently expressed repressor protein might be over-expressed in the non-inducible PEO-1 and PEO-6 cell lines (Jones, et al, 1985). Therefore, Northern analysis was used to measure induction of CYP1A1 mRNA by TCDD, after pre-treatment of the cells with cycloheximide.

## MATERIALS AND METHODS

### Cell Culture

Three ovarian cancer cell lines, PEO-1, PEO-4, and PEO-6, were grown in a-minimal essential media supplemented with 5% fetal calf serum and 1% antibiotic/antimycotic solution (Sigma). Cells were maintained in 150 $cm^2$ culture flasks in a humidified air/$CO_2$ incubator at 37°C. For RNA extractions, cells were passaged into 25 $cm^2$ culture flasks. At 60-85% confluency the cells were pre-treated with $10^{-5}$ M cycloheximide for 60 min, then dosed with $10^{-9}$ M TCDD for 5 hr. All chemicals were resuspended in DMSO; the final concentration of DMSO in the media was 0.2%.

### P4501A1 mRNA Levels

CYP1A1 mRNA levels were measured using a 1.2 Kb Pst1 fragment of the murine P1-450 cDNA purchased from ATCC (Rockville, Maryland). ß-Tubulin mRNA levels were measured using a 1.3 Kb EcoR1 fragment of human ß-tubulin cDNA, also purchased from ATCC. Total RNA was isolated by the guanidinium thiocyanate/acid phenol extraction method (Chomezynski, P. and Sacchi, N., 1987). Ten µg of total RNA was separated on a 1.2% agarose/1 M formaldehyde gel in 20 mM sodium phosphate, 2 mM CDTA, transferred onto nylon membrane by capillary action, and bound to the membrane by UV crosslinking. The cDNAs were labeled with a$^{32}$P-dCTP using a Random Primers DNA Labeling System (BRL) and added at 1-5 x $10^6$ CPM/ml hybridization solution (5X SSPE, 1% SDS, 10% dextran sulfate, 5X Denhardt's). Hybridization's were performed in roller bottles at 65°C using Rapid Hybridization buffer (Amersham) to block and hybridize. Bands were quantitated on a Betagen Betascope 603 blot analyzer imaging system and subsequently exposed to Kodak X-omat film. The data are expressed as the ratio of P-4501A1 message/ ß-tubulin message.

## RESULTS AND CONCLUSIONS

Table 1 summarizes the induction of steady-state CYP1A1 mRNA levels in the PEO-1, PEO-4, and PEO-6 ovarian cancer cell lines. The constitutive levels - both in the case of treatment with DMSO alone and treatment with $10^{-5}$ M cycloheximide alone - were relatively low in the 3 cell lines. Induction by TCDD alone was only significant in PEO-4 cells. In contrast, after co-treatment of the cells with TCDD and cycloheximide, induction was observed in all 3 cell lines, and the induced CYP1A1 mRNA levels were significantly higher than in cells treated with TCDD alone. It has previously been reported that cells treated with cycloheximide, a protein synthesis inhibitor, express higher levels of CYP1A1 mRNA and this may be due in part to the inhibition of endogenous proteins or polypeptides which repress constitutive CYP1A1 gene expression (Israel, et al, 1985 & 1983, Teifield et al, 1989, and Jones et al, 1985). The effects of cycloheximide on TCDD-induced CYP1A1 gene expression, however, were dependent on the cell line. The potentiation of TCDD by cycloheximide (T+C/TCDD) was higher in the non-responsive PEO-1 (4.2X) and PEO-6 (8.1X) cell lines than in the more TCDD-responsive PEO-4 cells (1.8X). However, the total amount of derepession (T+C/DMSO) was greatest in the PEO-6 cell line (13.8X). These data suggest that the intricate web of regulation for the CYP1A1 gene involves even more factors than the Ah receptor, DRE-binding efficiencies, and negative regulation.

The data reported in this study demonstrate that in the three human ovarian cancer cell lines, cycloheximide causes cell-specific effects on constitutive and TCDD-inducible CYP1A1 gene expression. Further studies are in progress to more clearly delineate the mechanisms associated with cell-specific-Ah-responsiveness. (Supported by the National Institute of Health ES03843)

| TREATMENT | CELL LINE (MEANS ± STANDARD DEVIATIONS) | | |
|---|---|---|---|
| | PEO-1 | PEO-4 | PEO-6 |
| DMSO | 0.018 ± 0.01 | 0.05 ± 0.008 | 0.025 ± 0.006 |
| TCDD | 0.012 ± 0.005 | 0.138 ± 0.019[a] | 0.043 ± 0.01 |
| CYCLO. | 0.018 ± 0.005 | 0.065 ± 0.013 | 0.055 ± 0.006 |
| T + C | 0.05 ± 0.008[a,b,c] | 0.243 ± 0.049[a,b,c] | 0.347 ± 0.05[a,b,c] |

**Table 1. Effects of Cycloheximide on TCDD-Induced CYP1A1 Gene Expression in Ovarian Carcinoma Cells.** Cells were pre-treated with DMSO or $10^{-5}$ M cycloheximide for 1 hr, then, within each group, cells were treated with DMSO or $10^{-8}$ M TCDD for an additional 5 hr. Total RNA was prepared and CYP1A1 mRNA levels were determined as described in the Materials and Methods section. The results are expressed as "means" ± "standard deviations" for 4 determinations for each treatment group.
[a]Statistically higher than cells treated with DMSO alone, as determined by ANOVA, ($p < 0.01$).
[b]Statistically higher than cells treated with TCDD alone, as determined by ANOVA, ($p < 0.01$).
[c]Statistically higher than cells treated with cycloheximide alone, as determined by ANOVA, ($p < 0.01$).

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