2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) AS A MONOOXYGENASE ENZYME INDUCER IN HUMAN CELL LINES: COMPARATIVE EFFECTS OF DIFFERENT ANTAGONISTS

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ABSTRACT

At a concentration of 10^{-9} M, TCDD significantly induced ethoxyresorufin-O-deethylase (EROD) activity in Hep-G2 (liver) and MCF-7 human breast cancer cell lines. The cells were also cotreated with TCDD and three different partial antagonists, namely α-naphthoflavone (α-NF), 6-methyl-1,3,8-trichlorodibenzofuran (MCDF) and Aroclor 1254 at concentrations of 10^{-8}, 10^{-7} and 10^{-6} M. α-NF and MCDF caused a concentration-dependent inhibition of TCDD-induced EROD activity in both cell lines. Aroclor 1254 was a less effective inhibitor of the induced enzyme activity and the effects were not concentration dependent.

INTRODUCTION

Recent studies in several laboratories have shown that a number of structurally-diverse compounds inhibited the TCDD-induced cytochrome P-450-dependent monooxygenases, aryl hydrocarbon hydroxylase (AHH) and EROD activity (1-10). For example, α-NF, Aroclor 1254 and MCDF inhibited the induction of AHH and EROD activity by TCDD in rat hepatoma H-4-IIE cells and in rodent liver microsomes. Some of these compounds also partially antagonized TCDD-induced teratogenicity, porphyria and immunotoxicity in C57BL/6 mice. This study reports the effects of α-NF, MCDF and Aroclor 1254 as antagonists of TCDD-induced EROD activity in transformed Hep-G2 human liver and MCF-7 human breast cancer cell lines.

MATERIALS AND METHODS

Treatment of Cells

Hep-G2 and MCF-7 cells were grown in MEM medium supplemented with 9.5 g/l Hepes buffer, 2.2 g/l sodium bicarbonate, 10mM sodium pyruvate, 6 μg/L insulin, 10% fetal calf serum, 50 μg/ml gentamicin sulfate, 50 μg/ml amphotericin B, 25 μg/ml penicillin and 25 μg/ml streptomycin. Stock cultures were maintained in 150 cm^2 tissue culture plates and incubated at 37°C in a humidified mixture of 5% CO_2 and 95% air under atmospheric pressure.

Enzyme Assays

Hep-G2 and MCF-7 were passaged to 25 cm^2 tissue culture flasks and treated with TCDD, α-NF, MCDF, Aroclor 1254, DMSO (control) or TCDD plus each inhibitor. Cells were harvested 24 h after treatment by manual scraping from the plate, centrifuged at 1000xg for 5 min (2°C) and resuspended in 200 μl Tris-sucrose. Aliquots of the cell suspension were assayed for EROD activity by the method Pohl and Fouts (11).

RESULTS AND DISCUSSION

Preliminary studies with both human cell lines showed that TCDD induced near maximum EROD activity at a concentration of 10^{-9} M. It was apparent from the results in Table 1 that the inducibility of this enzyme activity was higher in the Hep-G2 than in the MCF-7 Cells. Cotreatment of the cell lines with TCDD (10^{-9} M) and either 10^{-8}, 10^{-7} or 10^{-6} M concentrations of either MCDF or α-NF gave results which showed that the two antagonists caused a concentration-dependent decrease in EROD activity. The results were comparable to those previously reported using rat hepatoma H-4-II E cells (1,4).

Aroclor 1254, a commercial polychlorinated biphenyl mixture also inhibits TCDD-induced EROD activity in the human cell lines. In contrast to α-NF and MCDF, the antagonist activities were not concentration-dependent and Aroclor 1254 was clearly the least effective of the three antagonists. Future studies will utilize these cell lines as models for investigating the mechanism of action of the TCDD antagonists.

Table 1. Effects of TCDD and TCDD plus α-NF, MCDF or Aroclor 1254 as Inducers of EROD Activity in Hep-G2 liver and MCF-7 Breast Cancer Cells.

Treatment	EROD Activity(pmol/min/mg protein) HEP-G2	MCF-7
10^{-9} M TCDD	274 ± 16	78 ± 2
10^{-9} M TCDD + 10^{-8} M α-NF	208 ± 11[a]	67 ± 4[a]
10^{-9} M TCDD + 10^{-7} M α-NF	181 ± 12[a]	56.5 ± 16[a]
10^{-9} M TCDD + 10^{-6} M α-NF	12.5 ± 4[a]	2.5 ± 1[a]
10^{-6} M α-NF	6 ± 0[a]	0 ± 0[a]
10^{-9} M TCDD + 10^{-8} M MCDF	198 ± 6[a]	64.5 ± 2[a]
10^{-9} M TCDD + 10^{-7} M MCDF	149 ± 29[a]	30 ± 8[a]
10^{-9} M TCDD + 10^{-6} M MCDF	18.5 ± 1[a]	not detected[a]
10^{-6} M MCDF	19 ± 2[a]	1 ± 0[a]
10^{-9} M TCDD + 10^{-8} M Aroclor 1254	212 ± 3[a]	63 ± 0[a]
10^{-9} M TCDD + 10^{-7} M Aroclor 1254	166 ± 11[a]	61.5 ± 2[a]
10^{-9} M TCDD + 10^{-6} M Aroclor 1254	169 ± 9[a]	64 ± 0[a]
10^{-6} M Aroclor 1254	-0-	-0-
DMSO (solvent)	2.5 ± 0	not detected

[a]significantly lower ($p < 0.01$) than cells treated with 10^{-9} M TCDD alone.

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