α-NAPHTHOFLAVONE AND 1-AMINO-3,7,8-TRICHLORODIBENZO-p-DIOXIN AS 2,3,7,8-TCDD ANTAGONISTS IN RAT HEPATOMA H-4-II E CELLS

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ABSTRACT

Treatment of rat hepatoma H-4-II E cells with 10^{-9} M TCDD caused 90-100% of the maximum induction of ethoxyresorufin O-deethylase (EROD) activity. In contrast, at concentrations of 10^{-6} M, α-naphthoflavone (α-NF) and 1-amino-3,7,8-trichlorodibenzo-p-dioxin (NH_2-TrCDD) were inactive as inducers. Cotreatment of the cells with 10^{-9} M TCDD plus 10^{-8}-10^{-6} M α-NF or 10^{-8}-10^{-6} M NH_2-TrCDD resulted in a concentration-dependent decrease in the induction of EROD activity by TCDD; for example, 10^{-6} M concentrations of both antagonists causes a 66 and 45% decrease respectively in TCDD-induced EROD activities. α-NF (10^{-6} M) also caused a decrease in the formation of nuclear [^{3}H]-TCDD-receptor complexes and a reduction in P4501A1 mRNA levels (84 and 75% respectively). In contrast NH_2-TrCDD (10^{-6} M) caused only a 25% reduction in nuclear [^{3}H]-TCDD Ah receptor levels and a 19% decrease in P-4501A1 mRNA levels compared to cells treated with 10^{-9} M TCDD alone.

INTRODUCTION

Recent studies in this laboratory have shown that α-NF inhibited the induction of aryl hydrocarbon hydroxylase, EROD and P-4501A1 mRNA levels by TCDD in rat hepatoma H-4-II E cells (1). It was also shown the α-NF inhibited the accumulation of nuclear [^{3}H]-TCDD-receptor complexes. Double reciprocal plot analysis of the binding of [^{3}H]-TCDD with rat hepatic cytosol and different concentrations of α-NF gave results which suggested the α-NF acted as a competitive Ah receptor antagonist. It has previously been reported that NH_2-TrCDD also inhibited TCDD-induced responses (2) and this study will compare the antagonist activities of both α-NF and NH_2-TrCDD in rat hepatoma H-4-II E cells.

MATERIALS AND METHODS

Nuclear Extractions

H-4-II E cells were grown in α-MEM medium and treated with [^{3}H]-TCDD, α-NF, NH_2-TriCDD, [^{3}H]-TCDD + α-NF, [^{3}H]-TCDD + NH_2-TriCDD or DMSO (control). Cells were harvested one hour after treatment, centrifuged at 1000xg for 5 min (2°C), and resuspended in HEGD buffer. Nuclear extracts were prepared as described (3). Nuclear extracts were incubated with dextran-treated charcoal and then loaded onto linear sucrose gradients (5-25%). The gradients were centrifuged for 2.5 hours in a vertical tube rotor and then fractionated. [^{3}H]-TCDD in each fraction was determined by liquid scintillation counting. Baselines were obtained by coadministering a 200-fold excess of unlabelled TCDF.

mRNA Isolation

H-4-II E cells were grown in α-MEM medium and treated with TCDD, α-NF, NH_2-TriCDD, TCDD + α-NF, TCDD + NH_2-TriCDD, or DMSO (control). Cells were harvested 18 h after treatment by manual scraping from the plate, centrifuged at 1000xg for 5 min (2°C), and resuspended in phosphate buffered saline. Cells were lysed by the addition of guanidinium hydrochloride and the RNA isolated by phenol-chloroform extraction. DNA contamination was removed by selective precipitation of RNA with LiCl. Samples were then electrophoresed through an 0.8% agarose denaturing gel and transferred to a nylon membrane. The membrane was blocked and then probed with an 0.9 kb P-4501A1 cDNA fragment. Quantitation of bands was performed on a Betagen Betascope 603 Blot Analyzer. The P-4501A1 mRNA signal was standardized against a β-tubulin signal (1).

Enzyme Assays

H-4-II E cells were grown in α-MEM medium and treated with TCDD, α-NF, NH_2-TriCDD, TCDD + α-NF, TCDD + NH_2-TriCDD, or DMSO (control). Cells were harvested 24 h after treatment by manual scraping from the plate, centrifuged at 1000xg for 5 min (2° C) and resuspended in Tris-sucrose. Aliquots of the cell suspension were assayed for EROD activity by the method Pohl and Fouts (4).

RESULTS AND DISCUSSION

Table 1 summarizes the interactive effects of TCDD with α-NF and NH_2-TrCDD as inducers of EROD activities and both compounds exhibit a concentration-dependent

Table 1. α-NF and NH_2-TrCDD as TCDD Antagonists in Rat Hepatoma H-4-II E Cells.

Treatment	% Decrease in EROD Activity[a]
10^{-9} M TCDD + 10^{-8} M α-NF	44.7
10^{-9} M TCDD + 10^{-7} M α-NF	51.0
10^{-9} M TCDD + 10^{-6} M α-NF	66.2
10^{-9} M TCDD + 10^{-8} M NH_2-TrCDD	24.2
10^{-9} M TCDD + 10^{-7} M NH_2-TrCDD	38.9
10^{-9} M TCDD + 10^{-6} M NH_2-TrCDD	44.9

[a]compared to cell treated with 10^{-9} M TCDD alone; all the decreases were significant ($p < 0.01$).

antagonist effect on the induction response. In contrast, the results in Table 2 illustrate the α-NF causes a parallel concentration-dependent decrease in TCDD-induced P-4501A1 mRNA levels whereas at the highest dose of NH_2-TrCDD, only an 18.6% decrease in P-4501A1 mRNA levels were observed. The effects of α-NF and NH_2-TrCDD on nuclear

Table 2. Effects of α-NF and NH_2-TrCDD on TCDD-Induced P-4501A1 mRNA Levels in H-4-II E Cells

Treatment	% Decrease in P-4501A1 mRNA[a]
10^{-9} M TCDD + 10^{-8} M α-NF	26.8
10^{-9} M TCDD + 10^{-7} M α-NF	48.8
10^{-9} M TCDD + 10^{-6} M α-NF	74.9
10^{-9} M TCDD + 10^{-6} M NH_2-TrCDD	18.6

[a]compared to cells treated with $^{-9}$ M TCDD alone; all the decreases were significant ($p < 0.01$).

[^{3}H]-TCDD-receptor complexes were also compared (Table 3). As previously reported (1), α-NF caused a concentration-dependent decrease in nuclear [^{3}H]-TCDD-receptor levels whereas NH_2-TrCDD cause a maximum 25.54% reduction in these levels at the highest concentration (10^{-6} M). These results were similar to those previously reported for 6-methyl 1,3,8-trichlorodibenzofuran (3,5) and the data suggest that NH_2-TrCDD and MCDF may act as TCDD antagonists through a mechanism which is different from α-NF.

Table 3. Effects of α-NF and NH_2-TrCDD on the Accumulation of Nuclear [^{3}H]-TCDD-Ah Receptor Complexes in H-4-II E Cells.

Treatment	% Reduction in Nuclear Receptor Levels[a]
10^{-9} M TCDD + 10^{-8} M α-NF	39.6
10^{-9} M TCDD + 10^{-7} M α-NF	82.2
10^{-9} M TCDD + 10^{-6} M α-NF	84.3
10^{-9} M TCDD + 10^{-6} M NH_2-TrCDD	25.4

[a]compared to cells treated with [^{3}H]-TCDD alone; all the decreases were significant ($p < 0.01$).

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