3',4'-DIMETHOXYFLAVONE AS AN ARYL HYDROCARBON RECEPTOR ANTAGONIST IN BREAST CANCER CELLS

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Abstract

Previous studies have shown that several substituted flavonoids, including some 3'-substituted flavones, exhibit aryl hydrocarbon receptor (AhR) antagonist activities. We have been investigating the AhR agonist/antagonist activities of 3',4'-dimethoxyflavone (DMF) in estrogen receptor (ER)-positive MCF7 and T47D breast cancer cell lines. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) (1 nM) induced a 50- to 80-fold increase in CYP1A1-dependent ethoxyresorufin *O*-deethylase (EROD) activity in MCF-7 and T47D cells, whereas 0.1 to 10 μM 3',4'-DMF alone did not induce EROD activity. In cells cotreated with 1 nM TCDD plus 0.1 to 10 μM 3',4'-DMF, there was a concentration-dependent decrease in the induced response, with total inhibition at the 10 μM concentration. Gel mobility shift assays from rat liver cytosol and T47D cell nuclear extracts showed that 3',4'-DMF alone did not transform AhR to its nuclear binding form and inhibited TCDD-induced AhR transformation in both rat liver cytosol and T47D cell nuclear extracts. Therefore 3',4'-DMF exhibited AhR antagonist activity in breast cancer cells.

Introduction

Bioflavonoids and related synthetic analogs exhibit a broad spectrum of biological activities. Many of these compounds exhibit antimutagenic, anticarcinogenic activities[1]. Flavonoid-mediated effects are dependent on various factors including the structure of the compounds, the target organ or cell, and response. Previous studies in this laboratory have investigated the effects of various 3',4'-substituted flavones on aryl hydrocarbon receptor (AhR) mediated signal transduction pathways and 3'-methoxy-4'-nitroflavone was identified as a pure AhR antagonist[2]. This study describes research on 3',4'-dimethoxyflavone (DMF) as an AhR agonist/antagonist. Initial studies investigated the effects of 3',4'-DMF alone or in combination with TCDD on ethoxyresorufin-*O*-deethylase (EROD) activity, a P450 dependent enzyme in MCF-7 and T47D breast cancer cells. In addition, the effects of 3',4'-DMF alone or in combination with TCDD on transformation of the rat hepatic cytosol and T47D nuclear AhR complex were determined by gel electrophoretic mobility shift assays.

Methods and Materials

Cells, chemicals and biochemicals

TCDD was prepared in this laboratory (> 98% pure by chromatographic analysis) and 3',4'-DMF was commercially available (Aldrich Chemical Co.). MCF-7 and T47D human breast cancer cells were obtained from the American Type Culture Collection. The dioxin response element (DRE), and mutant DRE oligonucleotides were synthesized by the Gene Technologies Laboratory at

Texas A&M University. All other chemicals and biochemicals used in these studies were the highest quality available from commercial sources.

Cell growth

MCF-7 cells were grown as monolayer cultures in MEM supplemented with 10% fetal bovine serum plus $NaHCO_3$ (2.2 g/L), gentamycin (2.5 mg/L), penicillin/streptomycin (10,000 units/L and 10 mg/L), amphotericin B (1.25 mg/L) and 10 μg insulin. T47D cells were grown in αMEM supplemented with 2.2 g/l sodium bicarbonate, 5% fetal bovine serum and 10 ml antibiotic-antimycotic solution (Sigma). Cells were maintained in 150-cm^2 culture flasks in an air:carbon dioxide (95:5) atmosphere at 37°C.

Ethoxyresorufin *O*-deethylase (EROD) activity

Trypsinized cells were plated into 48-well tissue culture plates, allowed to reach 60% confluency, and treated with 1 nM TCDD, 0.1-10 μM 3',4'-DMF, or TCDD plus 3',4'-DMF for 24 hr. After 24 hr, cells were harvested and EROD activity was determined as described[3].

Preparation of nuclear extracts and rat liver cytosol, and gel mobility shift assays

Nuclear extracts from T47D cells and cytosol from rat liver were prepared as described[4]. Rat liver cytosol was incubated with different concentrations of the test compounds at 20°C for 2 hr. Ligand-induced AhR transformation of rat liver cytosol and T47D nuclear extracts was determined in gel mobility shift assays[4].

Statistical analysis

Statistical differences between different treatment groups were determined using Student's t test or ANOVA (Scheffe's) and the levels of significance were noted ($p < 0.05$). The results were expressed as means ± SE for at least three replicate determinations for each experiment.

Results and Discussion

Results summarized in Table show that 3',4'-DMF inhibited induction of EROD activity by TCDD at concentrations of 0.1 μM, 1 μM, and 10 μM in T47D cells and at concentrations of 1 μM and 10 μM in MCF-7 cells. The inhibitory response was concentration dependent manner, and 3', 4'-DMF alone did not induce nor inhibit EROD activity significantly in both cell lines. Results in Figure 1 show 3',4'-DMF alone (0.05 μM to 50 μM) did not transform the cytosolic AhR to its nuclear binding form and in combination with TCDD, 3',4'-DMF (5 μM and 50 μM) inhibited TCDD-induced transformation of the rat cytosolic AhR complex. Figure 2 also shows that similar results were observed for nuclear extracts from T47D cells. Treatment of the cells with 3',4'-DMF alone did not result in formation of an AhR complex and in combination with TCDD, 3',4'-DMF inhibited TCDD-induced transformation of the nuclear Ah receptor complex. Our results show that in common with other 3'-methoxy-substituted flavones 3',4'-DMF is an AhR antagonist in breast cancer cell lines[2,5].

Table 1. EROD activity in T47D and MCF7 breast cancer cells.

Treatment	EROD activity (pmol/min/mg)	
	T47D cells	MCF7 cells
DMSO	0.49± 0.11	0.56±0.03
TCDD(1nM)	43.2±7.00	26.58±2.36
3',4'-DMF 10-7M	0.78±0.08	1.05±0.06
3',4'-DMF 10-6M	0.39±0.09	0.63±0.02
3',4'-DMF 10-5M	0.36±0.14	0.44±0.08
T(1nM)+3',4'-DMF 10-7M	41.60±2.15*	25.84±0.04
T(1nM)+3',4'-DMF 10-6M	26.04±2.89*	12.85±1.50*
T(1nM)+3',4'-DMF 10-5M	0.55±0.04*	0.67±0.08*

* Significantly lower than observed for TCDD($p<0.05$).

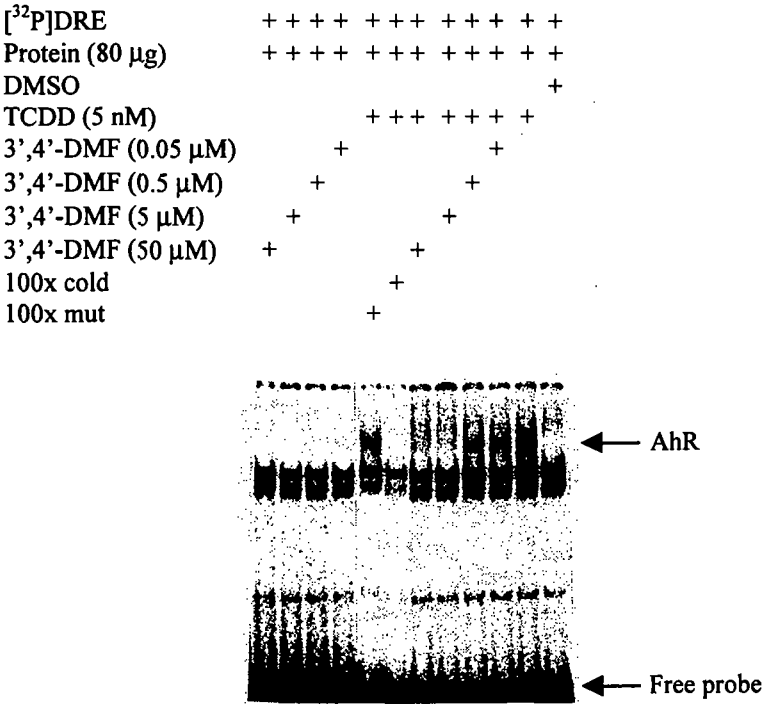

Figure 1. Ligand-induced Ah receptor transformation of rat liver cytosol.

[^{32}P]DRE	+	+	+	+	+	+
Nuclear extracts (5 μg)	+	+	+	+	+	+
Free probe						+
DMSO					+	
TCDD (5 nM)	+		+	+	+	
3',4'-DMF (5 μM)	+	+				
100x cold				+		
100x mut			+			

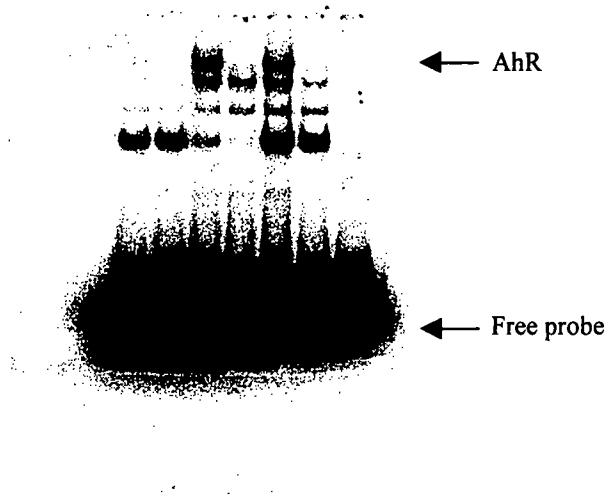

Figure 2. Ligand-induced formation of a nuclear Ah receptor complex in T47D cells.

Acknowledgments

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