INTERACTIONS OF RESVERATROL AND TCDD IN HUMAN BREAST CANCER CELLS

Jeong-Eun Lee and Stephen Safe

Texas A&M University, Department of Veterinary Physiology and Pharmacology, College Station, TX 77843-4466, USA

Abstract

Treatment of MCF-7 or T47D breast cancer cells with 1 nM 2,3,7,8-TCDD resulted in induction of CYP1A1 gene expression and related enzyme activities, and this was accompanied by ligand-induced formation of a nuclear aryl hydrocarbon receptor (AhR) complex. Resveratrol (up to 10 μM) alone did not induce CYP1A1 in breast cancer cells but formed a nuclear AhR complex; in combination with TCDD, resveratrol blocked CYP1A1 induction but did not affect nuclear AhR levels. In contrast, resveratrol did not block induction of reporter gene activity by TCDD in cells transfected with an Ah-responsive construct derived from the human CYP1A1 gene promoter. These results suggest that resveratrol does not act like a " classical " AhR antagonist.

Introduction

Resveratrol is a trihydroxystilbene that has been identified in wine, and several studies have linked the health promoting effects of modest wine consumption with the biochemical effects induced by resveratrol. At least one or more of three studies have reported that resveratrol interacts with the aryl hydrocarbon receptor (AhR) and blocks induction of CYP1A1 gene expression in various cancer cell lines[1,2,3]. We have reinvestigated the proposed AhR antagonist activities of resveratrol in MCF-7 and T47D breast cancer cell lines and our results confirm that resveratrol blocks induction of CYP1A1 gene/protein expression in breast cancer cell lines as previously reported[1,2,3]. However, the mechanism of this response may not directly involve inhibition of nuclear AhR action.

Methods and Materials

Cells, chemicals and biochemicals

TCDD was prepared in this laboratory (> 98% pure by chromatographic analysis), and resveratrol was commercially available (Sigma). 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 ug 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 resveratrol, or TCDD plus resveratrol for 24 hr. After 24 hr, cells were harvested and EROD activity was determined as described[4].

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[5]. 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[5].

Chloramphenicol acetyltransferase (CAT) activity

The plasmid pRNH11c contains the regulatory human CYP1A1 region from –1142 to +2434 fused to the bacterial CAT reporter gene. Cells were transiently transfected with pRNH11c, treated with 1 μM and 10 μM resveratrol alone or in combination with TCDD for 24-30 hr, and harvested. CAT assays were carried out as described[6]. CAT activity was standardized relative to β-galactosidase activity.

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

The activity of resveratrol as an inhibitor of AhR-mediated responses has previously been reported and some of the data are contradictory[1,2,3]. The results in Table 1 demonstrate that resveratrol inhibits induction of CYP1A1-dependent EROD activity by TCDD in breast cancer cells, and similar results have previously been reported. Ciolino and coworkers showed that after treatment of HepG2 cells with resveratrol alone, a nuclear AhR complex was not formed; however, resveratrol inhibited benzo[a]pyrene or TCDD-induced nuclear AhR complex as determined in gel mobility shift assays[2]. In contrast, another study showed that TCDD alone and in combination with resveratrol formed a nuclear AhR complex in T47D cells[3]. Our results in T47D and MCF-7 human breast cancer cells also show that resveratrol treatment results in formation of a nuclear AhR complex (Fig. 1). Previous studies demonstrate that TCDD and related AhR agonists readily transform the rat hepatic cytosolic AhR complex and forms a protein-DRE retarded band in gel shift assays; in this study treatment of cytosol with 5 nM TCDD gave a transformed retarded band (Fig. 2). Resveratrol also induced a dose-independent transformation of the cytosolic AhR complex, and we have previously observed similar results with 2,2',5,5'-tetrachlorobiphenyl, a compound with no AhR agonist activity suggesting that the significance of resveratrol interactions with the AhR must be carefully interpreted[7]. Moreover, in contrast to previous reports, our studies show that resveratrol does not significantly inhibit CAT activity induced by TCDD in MCF-7 and T47D cells transiently transfected with pRNH11c, an Ah-responsive construct containing the regulatory human CYP1A1 region from –1142 to +2434 fused to the bacterial CAT reporter gene (Table 2). The results contrast with previous studies showing that resveratrol exhibits AhR

antagonist activities with DRE-dependent constructs. We are now further investigating the mechanism of resveratrol-AhR interactions.

Acknowledgements

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Table 1. EROD activity in T47D and MCF-7 breast cancer cells.

Treatment	EROD Activity (pmol/min/mg)	
	T47D cells	MCF-7 cells
DMSO	0.85 ± 0.08	46.44 ± 2.82
TCDD (1 nM)	15.10 ± 0.40	472.07 ± 21.14
Resveratrol (10^{-7} M)	0.42 ± 0.04	50.83 ± 4.95
Resveratrol (10^{-6} M)	0.40 ± 0.05	49.43 ± 4.48
Resveratrol (10^{-5} M)	0.30 ± 0.05[a]	47.87 ± 0.55
T (1 nM) + Resveratrol (10^{-7} M)	14.41 ± 1.57[b]	397.99 ± 45.76[b]
T (1 nM) + Resveratrol (10^{-6} M)	13.00 ± 2.60	192.43 ± 21.08[b]
T (1 nM) + Resveratrol (10^{-5} M)	0.50 ± 0.06[b]	68.85 ± 9.70[b]

[a] Significantly lower than observed for DMSO ($p<0.05$).
[b] Significantly lower than observed for TCDD ($p<0.05$).

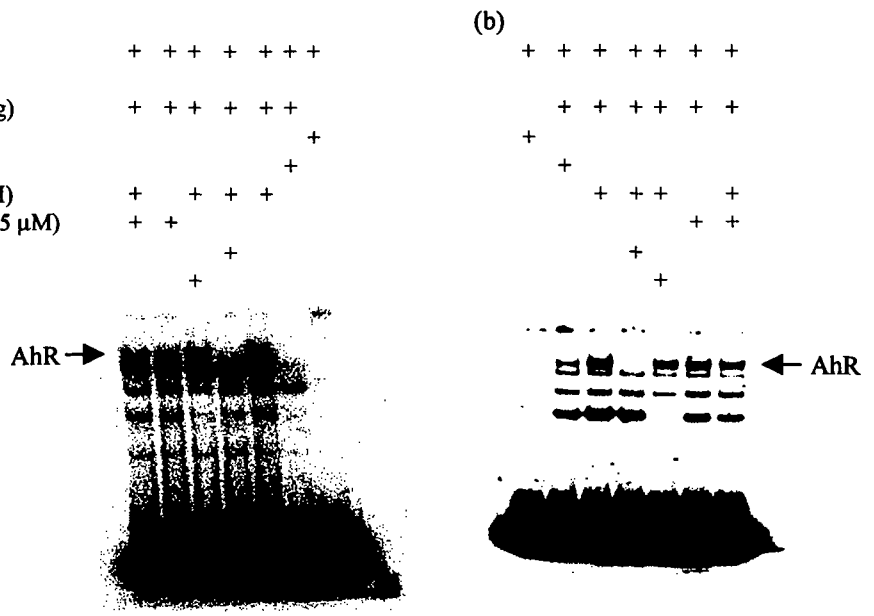

Figure 1. Ligand-induced formation of a nuclear AhR complex in T47D (a) and MCF-7 (b) cells.

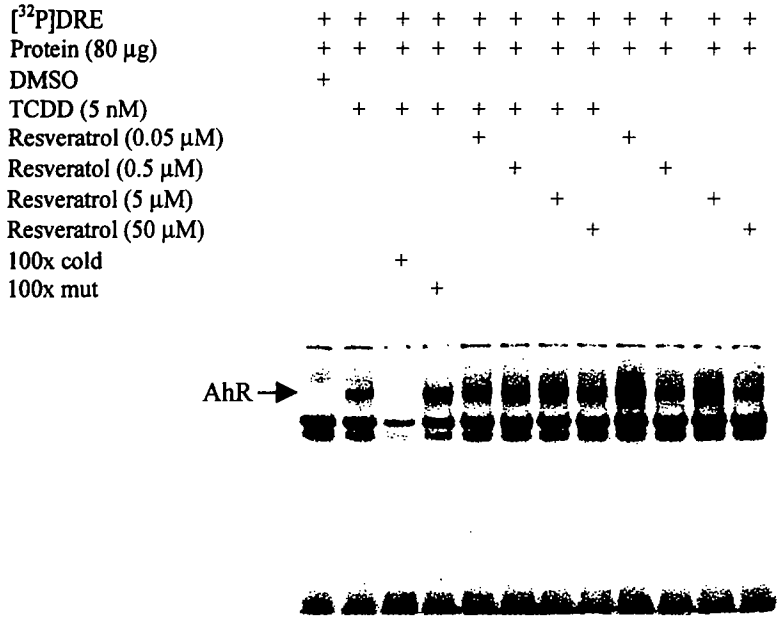

Figure 2. Ligand-induced AhR transformation of rat liver cytosol.

Table 2. CAT activity in T47D and MCF-7 breast cancer cells transfected with pRNH11c.

Treatment	Relative CAT activity (%)	
	T47D cells	MCF-7 cells
DMSO	100.00 ± 11.76	100.00 ± 5.96
TCDD(1 nM)	739.06 ± 47.47	379.15 ± 37.21
Resveratrol(10-6 M)	77.92 ± 11.58	123.59 ± 8.60
Resveratrol (10-5 M)	116.62 ± 17.98	50.14 ± 4.44
T (1 nM) + Resveratrol (10^{-6} M)	534.82 ± 49.47	373.47 ± 60.90
T (1 nM) + Resveratrol (10^{-5} M)	783.10 ± 53.89	285.99 ± 24.57

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