EFFECT OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) ON 17ß-ESTRADIOL-INDUCED CATHEPSIN D PROMOTER FUNCTION

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Abstract

Cytosolic levels of procathepsin D and cathepsin D (CAT-D) are used as negative prognostic indicators of disease-free survival in women suffering from breast cancer. MCF-7 human breast cancer cells were co-transfected with the pBC12/pL/pac plasmid containing the CAT-D promoter and a human estrogen receptor (hER) expression plasmid. Treatment with 10 nM 17ß-estradiol (E2) resulted in a 844± 89% increase in secreted alkaline phosphatase (AP) activity compared to control cells; 100± 30%. Addition of 10 nM TCDD 24 hr after E2 treatment resulted in a time-dependent decrease in E2-induced AP activity and was significantly reduced 6 hr (321± 30% control) after addition of TCDD. Treatment of co-transfected cells with 10 nM TCDD alone resulted in an unexpected increase in AP activity (708± 50% control). Hepa 1c1c7, wild type and class II mutant cells which lack functional nuclear aryl hydrocarbon (Ah) receptor were used to determine the role of the Ah receptor in these effects. Results from these experiments indicate a) TCDD-induced decrease in E2-induced AP activity is mediated by the Ah receptor and b) the increase in AP activity caused by 10 nM TCDD alone is not mediated through the Ah receptor.

Introduction

Cathepsin D protein is hypersecreted by MCF-7 human breast cancer cells upon treatment with E2 [1]. It has been suggested that this protease may play a role in tumor metastasis and is used clinically as a negative prognostic indicator of disease free survival for women with breast cancer [2]. E2 is known to induce CAT-D gene expression in MCF-7 cells via the E2-estrogen receptor complex [3]. It was previously shown in our laboratory that TCDD can cause inhibition of the E2-induced secretion of CAT-D protein in MCF-7 cells [4]. TCDD and other related polyaromatic hydrocarbons (PAH's) mediate their biochemical effects via the Ah receptor complex [5]. The signal transduction

pathway for transcriptional regulation in Ah receptor mediated responses is very similar to that described for steroid hormones. In this study we have investigated the effect of TCDD and determined the role of a functional nuclear Ah receptor on CAT-D promoter activity using transient transfection assays in three different cell lines.

Materials and Methods

Treatment and cells: Human breast cancer MCF-7 cell line was obtained from the American Type Culture Collection (Rockville, MD). Cells were maintained in DME/F12 medium with phenol red and supplemented with 5% fetal bovine serum (FBS) plus 1% antibiotic-antimycotic solution (Sigma) in an air:carbon dioxide (95:5) atmosphere at 37°C. Cells were grown in DME/F12 without phenol red and in FBS twice stripped with 10% dextran coated charcoal for 2 days before harvesting.

Transient transfection assay: Transient transfection assays were performed using calcium phosphate as described [6]. Cells were treated with 10 nM E2 (Sigma) 2 hr after co-transfection with pBC12/pL/pac and hER plasmids; 10 nM TCDD was added 24 hr after E2-treatment. At appropriate times the treatment cells were harvested and assayed for AP activity. In E2 and DMSO (control, vehicle) treated cells the media was obtained 48 hr after E2 or DMSO treatment respectively. The media from the positive and negative control cells were obtained 50 hr after transfection with the pBC12/RSV/pac and pBC12/S1/pac plasmids respectively.

Alkaline phosphatase assay: Alkaline phosphatase activity was measured as described [7].

Plasmid constructs: The pBC12/pL/pac plasmid containing the CAT-D promoter (-296 to +57) fused to an alkaline phosphatase gene was used to study the promoter activity. The pBC12/S1/pac plasmid containing no promoter and the pBC12/RSV/pac plasmid containing a Rous Sarcoma Virus promoter fused to the alkaline phosphatase gene was used as negative and positive controls respectively. The above plasmids were generous gifts from Dr. Andrej Hasilik, Munster, Germany (with permission from Dr. Cullen, B.R., Duke University, Durham, North Carolina). The hER plasmid containing the human estrogen receptor expression vector was a gift from Dr. Ming-Jer Tsai, Baylor College of Medicine, Houston, Texas.

Results and Discussion

The major objective of this study was to determine the role of the Ah receptor in mediating the decrease in E2-induced CAT-D promoter activity by TCDD. Initial studies focused on the time-dependent effects of 10 nM TCDD on E2-induced AP activity in MCF-7 human breast cancer cells which contain functional nuclear Ah and estrogen receptor-complexes. In MCF-7 cells treated with 10 nM E2 there was an 8-fold increase in AP activity when compared to DMSO treated cells. Addition of 10 nM TCDD 24 hr after E2

treatment decreased the AP activity as early as 2 hr after TCDD treatment and was statistically significant from 6 hr to 24 hr after addition of TCDD (Table I). Treatment of MCF-7 cells for 12 hr with 10 nM TCDD alone resulted in an unexpected 7-fold increase in AP activity. This increase was only seen in cells co-transfected with hER plasmid containing the human estrogen receptor (Table I).

In similar studies carried out with wild-type Ah responsive and non-responsive class II mutant cells, it was shown that the increase in AP activity when these cells were co-transfected with the hER plasmid and treated with 10 nM TCDD alone, was observed in both the mutant and wild type cells (Table II). This indicated that the presence of a functional nuclear Ah receptor was not required for this effect. In contrast the TCDD-induced decrease in E2-induced AP activity was only observed in the wild type and not in the class II mutant cells (refer Table II). This data indicates that the TCDD-induced decrease of the E2-induced CAT-D promoter activity requires a functional nuclear Ah receptor. (Supported by the National Institutes of Health ES04176)

Table I. Time-dependent effects of 10 nM TCDD on E2-induced CAT-D promoter activity in MCF-7 human breast cancer cells.

Treatment (time, hr)	AP activity as % control	Treatment (time, hr)	AP activity as % control
DMSO (48)	100±1%	E2+TCDD (6) **	311±30%
TCDD only (12)	115±30%	E2+TCDD (12) **	245±28%
TCDD only [a] (12)	708±50%	E2+TCDD (18) **	165±27%
E2 (48)	845±89% *	E2+TCDD (24) **	153±13%
E2+TCDD (1)	782±87% *	S1	53±13%
E2+TCDD (2)	629±30% *	RSV	1533±240%
E2+TCDD (4)	515±47% *		

All groups were co-transfected with the hER and pBC/pL/pac plasmids except 'TCDD only' which was not co-transfected with the hER plasmid. The S1 and RSV groups were transfected with the negative and positive control plasmids respectively. The E2 concentration was 10 nM.
* Significantly higher than ($p<0.05$) DMSO treated cells
** Significantly lower than ($p<0.05$) E2 treated cells

Table II. Time-dependent effects of 10 nM TCDD on E2-induced CAT-D promoter activity in Hepa 1c1c7 wild type and class II mutant, mouse hepatoma cell lines.

Treatment (time, hr)	Hepa 1c1c7 (wt) AP activity as % control	Hepa 1c1c7 (class II mut) AP activity as % control
DMSO (48)	100±1%	100±14%
TCDD only (12)	96±3%	74±19%
E2 (48)	690±30%**	640±100%**
TCDD (2)	346±48%**	249±31%**
TCDD (6)	366±49%**	313±29%**
TCDD (12)	451±16%**	435±66%**
TCDD (24)	446±33%**	500±72%**
E2+TCDD (2)	316±38%**	501±70%**
E2+TCDD (6)	435±51%**	657±47%**
E2+TCDD (12)	268±9%*	595±72%**
E2+TCDD (24)	115±16%*	601±99%**
S1	37±3%	22±14%
RSV	1250±17%	860±72%

All groups were co-transfected with the hER and pBC/pL/pac plasmids except 'TCDD only' which was not co-transfected with the hER plasmid. The S1 and RSV groups were transfected with the negative and positive control plasmids respectively. The E2 concentration was 10 nM.
* Significantly lower ($p<0.05$) than E2 treated cells.
** Significantly higher ($p<0.05$) than DMSO treated cells

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