COMPARATIVE EFFECTS OF 2,3,7,8,-TETRACHLORODIBENZO-*P*-DIOXIN AND RETINOIC ACID IN MCF-7 BREAST CANCER CELLS

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Abstract
Previous studies have shown that retinoic acid(RA) inhibited the growth of MCF-7 human breast cancer cells, suppressed both the constitutive and estrogen-induced pS2 gene and transforming growth factor-α gene expression. The antiestrogenic and antiproliferative properties of RA and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) are similar in MCF-7 cells and their effects and interactions were investigated. Both RA and TCDD inhibited 17β-estradiol-induced cell growth and ^{3}H-thymidine incorporation in MCF-7 cells. In cotreatment studies, the effects of RA Plus TCDD on estradiol-induced cell proliferation and thymidine incorporation were additive. Velocity sedimentation analysis of nuclear estrogen receptor(ER) binding activity indicated a time-dependent decrease in ER levels; 24 hr after RA treatment, ER levels were decreased by 32%. Inhibition of nuclear ER levels elicited by RA plus TCDD were also additive. In a parallel experiment, the RA-induced effects on binding of nuclear ER protein from the treated cells with estrogen responsive element (ERE) were determined using a gel mobility shift assay. The results showed that there was a 45% decrease in ERE binding within 1 hr after treatment with RA and this response persisted for up to 24 hr, suggesting that decrease of nuclear ER protein or/and ER-ERE binding affinity may be involved. Comparable results were also obtained for TCDD.

Introduction
Retinoids are known to inhibit mammary carcinogenesis in rodents[1-3]. Moreover, retinoids or retinoids in combination with antiestrogens inhibit the proliferation of human breast cancer cells. The inhibition of breast cancer cell proliferation elicited by retinoids is related to estrogen recepror status of the cells[4-7]. This highlights an intriguing possibility that retinoids may interact with steroid hormone receptors in modulating cellular growth. Several studies have suggested that retinoids may act as antiestrogens[8-9]. Koga and Sutherland[7] reported that RA did not affect estradiol-induced growth stimulation in MCF-7 cells at concentrations from 10^{-11} to 10^{-8} M. Recent studies[10] using bioluminescence detection demonstrated that RA inhibited the estrogen-dependent induction of luciferase gene transcription. The results also suggested that changes in the characteristics of cellular ERs may be related to the effects of retinoids on ER-positive breast cancer cells. 2,3,7,8-TCDD, an environmental contaminant, also inhibits the growth of human breast cancer cells and downregulates the nuclear ER[11-13]. These results suggested that there may be some common features in the mechanism of action of

retinoids and TCDD in breast cancer cell lines. In this study, the effects of RA, TCDD and their combination on the growth of MCF-7 cells was investigated and their effects on the ER were also determined.

Maretials and methods

Cells and cell culture: MCF-7 cells were originally obtained from ATCC and routinely maintained in DME/F12 with phenol red, 6mg/L bovine insulin and 5% fetal bovine serum. All-trans retinoic acid (Sigma) and TCDD were dissolved in DMSO, 17ß-estradiol in ethanol. Cell proliferation experiments were performed as described previously[14].

^{3}H-Thymidine incorporation assay: Incorporation of ^{3}H-thymidine into the MCF-7 cells were determined as described[14].

Preparation of nuclear extracts: For nuclear ER isolation, MCF-7 cells were seeded in T-150 culture flasks($15x10^6$ cells/flask) in normal media. Two days before harvest, cells were changed to DME/F12 phenol red-free media. Chemicals were added for different periods of time before harvest. Nuclear extracts were prepared from the treated cells according to the described procedure[11].

Velocity sedimentation analysis: Treated cells were incubated with [^{3}H]-estradiol(10^{-9} M) 1hr before harvest. Aliquots of nuclear extract were layered on linear sucrose gradients(5%-25%) prepared in TEGD buffer as described[11].

Estrogen receptor DNA binding assay: Binding activity of nuclear ER protein to its responsive DNA sequence(ERE) was determined by gel mobility shift assay[15]. The [^{32}P]-ERE used in this assay was a complementary pair of oligonucleotides containing the sequence 5'-GTCCAAAGTCAGGTCACAGTGACCTGATCAAAGTT-3' corresponding to the ERE identified by Kumar and Chambon[16].

Results and discussion

Growth inhibition of RA and TCDD on MCF-7 cells: In this stidy, RA caused a significant inhibition of the growth of MCF-7 cells at concentrations of 10^{-7} and 10^{-6} M. Both RA and TCDD inhibited 17ß-estradiol induced cell growth. In cotreatment studies, the effects of RA plus TCDD (combined) on 17ß-estradiol-induced cell proliferation were additive(Fig.1).

Table1. Effects of RA and TCDD on the incorporation of ^{3}H-thymidine in MCF-7 cells*

Treatment	^{3}H-thymidine incorp. (DPM/mg protein x10^3)	% of control
Control	687.2±41.0	100
10^{-8}M TCDD	800.7±176.1	116.5
10^{-9}M E_2	4208.2±242.6 **	612.3
10^{-6}M RA	669.8±187.6	97.5.
E_2+RA	2575.1±453.7***	374.7
E_2+TCDD	2327.1±264.2***	338.6
E_2+RA+TCDD	974.8±105.2***	141.8

* Data are obtained from three separative determinations
** Significantly different from control group($p<0.05$).
*** Significantly different from E_2 group($p<0.05$).

Table 2: Time-dependent effects of RA(10^{-6}M) on nuclear ER levels in MCF-7 cells*

treat.ment RA (hr)	Nuclear ER levels (fmol/mg protein)	% of control
0	554.8±41.0	100
1	382.9±28.9**	69.0
3	496.6±46.1	89.0
6	447.3±21.0**	80.6
12	442.7±12.3**	79.8
24	376.6±27.1**	67.9

* Data are mean±SD from three separate determinations.
** Significantly different from control group ($p<0.05$).

Inhibition of ^{3}H-thymidine incorporation in MCF-7 cells: Both RA(10^{-6} M) and TCDD(10^{-8} M) inhibited estradiol-induced thymidine incorporation in MCF-7 cells (Table1). Cotreatment of MCF-7 cells with TCDD plus RA resulted in additive inhibition of 17β-estradiol-induced thymidine incorporation.

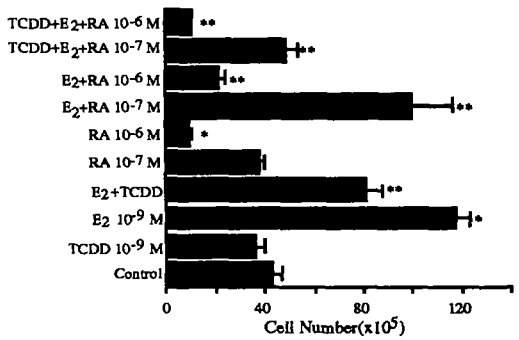

Fig.1: Effects of RA and TCDD on the growth of MCF-7 cells. Cells were treated with reagents as indicated for 6 days. Cell number were determined using coulter counter. Data are means±SD obtained from three separate determinations.
* significantly differtent from control group($p<0.05$).
** significantly different from E2 group($p<0.05$)

Effects of RA and TCDD on nuclear ER profile: Velocity sedimentation analysis of nuclear ER binding activity showed that at a 10^{-6} M concentration of RA, there was a time-dependent decrease in nuclear ER levels and 24 hr after RA treatment, the nuclear ER levels were decreased by 32% (Table 2). Cotreatment of MCF-7 cells with both RA(10^{-6} M) and TCDD(10^{-9} M) for 4 hr also resulted in decreased ER levels and the data indicated that the combined effects of both agents were additive(Fig.2).

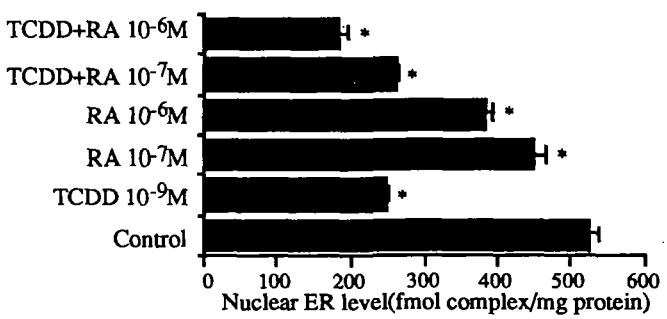

Fig2: Effects of RA and TCDD on nuclear ER levels in MCF-7 cells
Subconfluent MCF-7 cells were treated with TCDD or/and RA for 4 hr.
Nuclear ER levels were determined using velocity sedimentation analysis.
Data are obtained from three separate determinations.
* significantly different from control group($p<0.05$).

Effects of RA on nuclear ER-ERE binding: The ERE gel shift assay showed that after treatment of MCF-7 cells with 10^{-6} M RA, there was a significant decrease in the ER-ERE binding over a 24 hr period (Fig.3). This indicated that there was either a decrease in nuclear ER protein or/and a decreased affinity of ER for the [^{32}P]-ERE. Inhibition of ER-

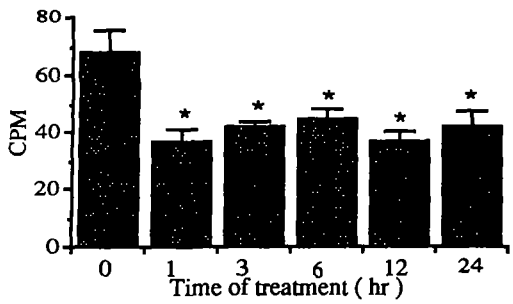

Fig.3: Effects of RA(10^{-6}M) on nuclear ER-ERE binding in MCF-7 cells.Cells were treated with 10^{-6} M RA for 0-24 hr. Nuclear ER-ERE binding activity was determined using a gel shift assay. The radioactivity of the ER-ERE retarded band is shown in the figure. Data are obtained from three separate determinations.
* significantly lower than control group($p<0.05$).

ERE binding was observed as early as 1 hr after treatment with 10^{-6} M RA and this response persisted for up to 24 hr. Comparable results were also obtained from TCDD. The results of this study confirmed that both RA and TCDD cause a similar spectrum of responses in MCF-7 cells. In cotreatment studies, their combined responses were additive. (Supported by the National Institutes of Health, ES04176)

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