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EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) ON CELL GROWTH AND THE SECRETION OF THE ESTROGEN-INDUCED 34-, 52- AND 160-kDa PROTEINS IN HUMAN BREAST CANCER CELLS

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Summary—The effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the growth of estrogen-responsive MCF-7 human breast cancer cells in the presence of 17β-estradiol was determined. After treatment with 17β-estradiol (1 nM), TCDD (10 nM) and 17β-estradiol (1 nM) plus TCDD (10 nM) the cells were monitored daily for cell growth and DNA content for 7 days. The results showed that TCDD inhibited cell proliferation and DNA content of untreated cells and inhibited the 17β-estradiol-stimulated cell proliferation and increase in cellular DNA content. In contrast, TCDD did not effect the growth of estrogen non-responsive MDA-MB-231 human breast cancer cells. TCDD (0.1–10 nM) also caused a concentration-dependent decrease in the 17β-estradiol-induced proliferation in MCF-7 cells. The effects of TCDD on the 17β-estradiol-induced secretion of the 52-kDa protein (i.e. procathepsin D), the 34-kDa (cathepsin D) and 160-kDa proteins were also determined in the MCF-7 and MDA-MB-231 human breast cancer cell lines. The levels of the proteins were determined by autoradiographic analysis of the incorporation of [^{35}S]methionine into the secreted proteins which were separated by denaturing sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Treatment of MCF-7 cells with 17β-estradiol (1 nM), TCDD (10 and 100 nM) and 17β-estradiol (1 nM) plus TCDD (10 nM) resulted in levels of the 52-kDa protein which were 497, 63.6, 98.1 and 66.3%, respectively, of the corresponding levels observed in control (untreated) cells. Using the same concentrations, the levels of the 34-kDa protein secreted into the media were 372, 42.3, 64.0 and 43.8% of control values, respectively, and the corresponding levels of the 160-kDa protein were 381, 52.9, 71.2 and 76.6% of the control values, respectively. In contrast, treatment of MDA-MB-231 cells with 17β-estradiol (1 nM), TCDD (10 and 100 nM) and 17β-estradiol (1 nM) plus TCDD (10 nM) resulted in a 31–39% reduction in the secretion of the 52-kDa protein however these effects were not statistically different from the control values. In addition, the treatments did not cause any significant effects on the secretion of the 34- and 160-kDa proteins by MDA-MB-231 cells. These results clearly confirm and extend the range of antiestrogenic effects caused by TCDD in estrogen-responsive MCF-7 cells and indicate that the MDA-MB-231 cells are not responsive to the antiestrogenic effects of TCDD.

INTRODUCTION

MCF-7 human breast cancer cells contain relatively high levels of the estrogen receptor and these cells have been extensively utilized to investigate the cellular and molecular mechanisms of estrogen-stimulated gene transcription and growth [1–7]. The treatment of MCF-7 cells with estrogens results in increased growth [2, 6, 7] which is accompanied by the induction of a number of enzymes [8–10] and the enhanced secretion of several proteins [11–16]. One of the secreted proteins, designated the 52-kDa protein, is an aspartyl protease which has been identified as procathepsin D [1, 11, 12, 14, 17]. This protein acts as a mitogenic factor and stimulates the growth of MCF-7 cells. In addition, this enzyme degrades the extracellular matrix from bovine endothelial cells and this proteolytic property may be an important factor in facilitating tumor invasion of these cells in acidic environments. Treatment of MCF-7 cells also enhances the secretion of a 34- and 160-kDa protein [19] and the former protein has been identified as the mature form of the enzyme cathespin D. It has recently been reported that high levels of either the 34- or 52-kDa proteins in human breast cancer tissue

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were significant predictors of reduced disease-free survival in some groups of patients [20, 21].

Several studies have reported that triphenylethylene-derived antiestrogens such as tamoxifen inhibit several estrogen-stimulated processes in MCF-7 cells including growth and the secretion of several proteins including the 34-, 52- and 160-kDa proteins [1–6, 14, 19, 22–24]. Recent studies have reported that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds also exhibit several antiestrogenic responses in the rodent uterus [25–29] and MCF-7 cells in culture [30, 31]. For example, in the rat uterus, TCDD caused a dose-dependent decrease in constitutive and estrogen-induced nuclear and cytosolic estrogen and progesterone receptor levels, and uterine peroxidase activity [26–29]. TCDD also inhibits estrogen-induced increases in rat uterine wet weight and exhibits comparable antiestrogenic activities in mice [25]. It has also been reported that estrogen-responsive MCF-7 human breast cancer cells contain the TCDD or aryl hydrocarbon (Ah) receptor protein and are also responsive to the effects of TCDD and related compounds on the induction of CYP1A1 gene expression [32, 33]. Gierthy and co-workers have reported that TCDD inhibited cell growth, postconfluent focus production and the estrogen-induced secretion of tissue plasminogen activator activity in MCF-7 cells [30, 31].

This paper reports the effects of TCDD on the constitutive and 17β-estradiol-stimulated growth and the secretion of the 34,- 52- and 160-kDa proteins in MCF-7 human breast cancer cells in culture. In addition, parallel experiments using estrogen and TCDD non-responsive MDA-MB-231 cells were also carried out.

EXPERIMENTAL

Chemicals and biochemicals

The TCDD ($>98\%$ purity) was synthesized in this laboratory. The radiolabeled [^{14}C]methylated protein kit and [^{35}S]methionine (9.52 mCi/ml) were purchased from Amersham and New England Nuclear, respectively. All other chemicals and biochemicals were the highest purity available from commercial sources.

Cell proliferation assay

MCF-7 and MDA-MB-231 human breast cancer cell lines, were obtained from the ATCC. Stock cultures were maintained in T-150 Corning plastic culture flasks, on RPMI 1640 medium, supplemented with 5% fetal calf serum. Stock cultures were conditioned to grow in RPMI 1640 medium supplemented with 3% dextran-coated charcoal treated fetal calf serum. Conditioned MCF-7 cells were suspended with trypsin and seeded into T-25 plastic tissue culture flasks at a concentration of 5×10^4 cells/flask. The cultures were allowed to incubate at 37°C and 5% CO_2 for 24 h. The medium was then removed, the cultures were washed twice with phosphate-buffered saline (PBS) and the cultures were refed with medium containing 10 nM TCDD, 1 nM 17β-estradiol or 1 nM 17β-estradiol plus 10 nM TCDD. DMSO was used as the vehicle for the compounds as well as the control studies. The cultures were refed daily thereafter. Upon termination of the experiment, the medium was removed and the cells were washed twice with PBS. The cultures were then trypsinized, the cells were counted and the numbers were recorded. The cells were centrifuged for 10 min at 3000 rpm. The trypsin was removed and replaced with TEN buffer. The cells were sonicated for 45 s and then the protein and DNA content were determined.

Cell culture conditions for analysis of the 34-, 52- and 160-kDa proteins

The cells were maintained in a monolayer with RPMI 1640 media, supplemented with 83 μg/l insulin and 5% fetal calf serum (FCS). On day -7 the cells were washed twice with PBS and passaged into RPMI 1640 medium containing 83 μg/l insulin and 3% FCS. On day -2 the cells were washed twice with PBS and were passaged into Corning 24-well culture dishes with RPMI 1640 medium containing 3% FCS, which had been treated with dextran-coated charcoal (FCS-DCC), and no insulin, at a concentration of 50,000 cells/well in 1 ml of media. On day 0 the medium was removed, and the cells were rinsed twice with PBS. The medium was then replaced with RPMI 1640 containing 3% FCS-DCC, no insulin and one of the following chemicals; 1 nM 17β-estradiol, 10 nM TCDD, 100 nM TCDD or 1 nM 17β-estradiol + 10 nM TCDD. The dose–response effects of TCDD (10^{-10}–10^{-13} M) were also determined using a comparable protocol. The cells were allowed to incubate for 48 h. The medium was changed after 24 h; 6 h prior to the termination of the experiment, the medium was removed, the cells were washed twice with PBS

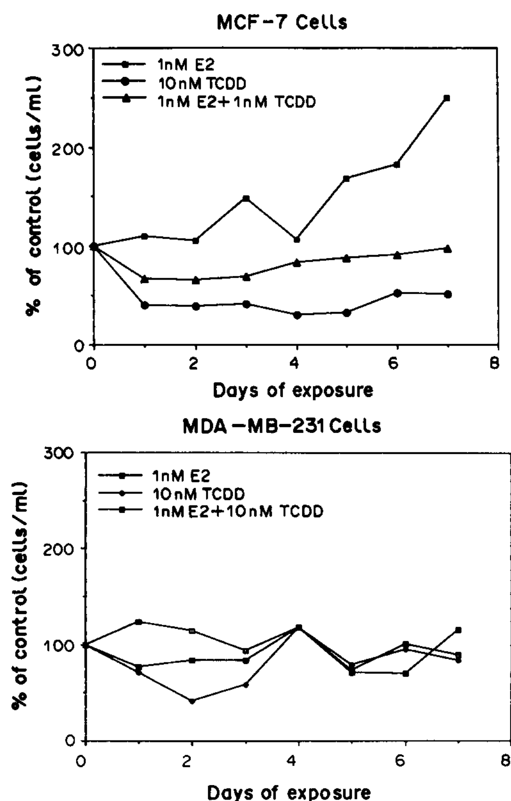

Fig. 1. The effects of 1 nM 17β-estradiol, 10 nM TCDD and 1 nM 17β-estradiol plus 10 nM TCDD on the growth of MCF-7 (top) and MDA-MB-231 (bottom) cells as a % of (vehicle) control cell growth. The growth conditions are summarized in the Experimental section. From days 5 to 7, TCDD significantly ($P < 0.05$) inhibited the constitutive and 17β-estradiol-induced growth of MCF-7 cells compared to the control cells whereas no effects were observed in the MDA-MB-231 cells.

and the medium was replaced with methionine-deficient RPMI 1640 containing 3% FCS-DCC, the same chemicals and 2 μl [^{35}S]methionine (10 mCi/ml) for a total volume of 200 μl. After 6 h the medium was removed, 10 μl was counted and the remaining medium was used for analysis of secreted proteins.

The secreted proteins were analyzed by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis; 30 μl of the medium was mixed with an equal volume of buffer containing SDS, β-mercaptoethanol, glycerol, Tris and bromophenol blue. The samples were heated for 2 min in a boiling water bath and 50 μl of each sample was loaded onto a 1.5 mm thick 12% acrylamide slab gel (containing 0.075% bis-acrylamide) with a 3% stacking gel (containing 0.15% bisacrylamide). The gels were run at 50 mV overnight and processed for fluorography (using Enhance: Dupont) and exposed to Kodak X-OMAT film for 10 days at −80°C. The molecular weights of the proteins were estimated by their mobility relative to a set of standard proteins with known molecular weights.

The incorporation of [^{35}S]methionine into the 34-, 52- and 160-kDa proteins was estimated by scanning the films using a Zeineh Soft Laser Scanning Densitometer (model SLR-20/10) from Biomedical Instruments Inc. The percentage of the area of the scans due to the particular protein was then compared between the experimental and control samples and the results are presented as means ± SD for at least 3 samples per treatment group.

RESULTS

Figure 1 summarizes the time-course effects of 17β-estradiol (1 nM), TCDD (10 nM) and 17β-estradiol plus TCDD on the proliferation of MCF-7 and MDA-MB-231 human breast cancer cells in culture. 17β-Estradiol significantly increased proliferation of the MCF-7 cells whereas treatment with TCDD (10 nM) decreased cell proliferation and significantly inhibited 17β-estradiol-induced proliferation of the MCF-7 cells from 5 to 7 days after initial treatment. In contrast, the growth curves for MDA-MB-231 cells treated with 17β-estradiol (1 nM), TCDD (10 nM) or 17β-estradiol plus TCDD were not significantly different from the control cells after 7 days. Comparable results were observed for the cellular DNA levels in these cell lines (data not shown). The results illustrated in Fig. 2 show that 17β-estradiol (1 nM) caused an increase in the growth of MCF-7 cells in culture and TCDD (0.1, 1.0, 5.0 and 10 nM) caused a concentration-dependent decrease in 17β-estradiol-enhanced growth in

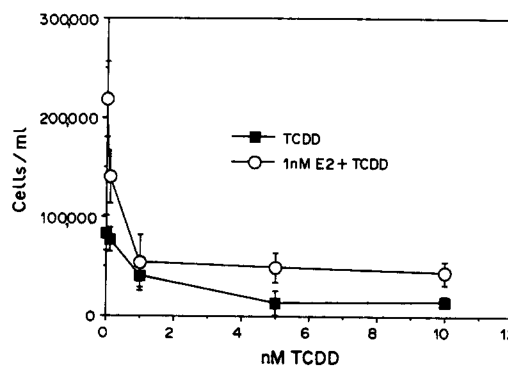

Fig. 2. The concentration-dependent effects of TCDD (0.1, 1, 5 and 10 nM) in the presence or absence of 17β-estradiol (1 nM) on the growth of MCF-7 cells in culture. The growth conditions are summarized in the Experimental section and the data in the figure summarize the cell number 7 days after initial treatment.

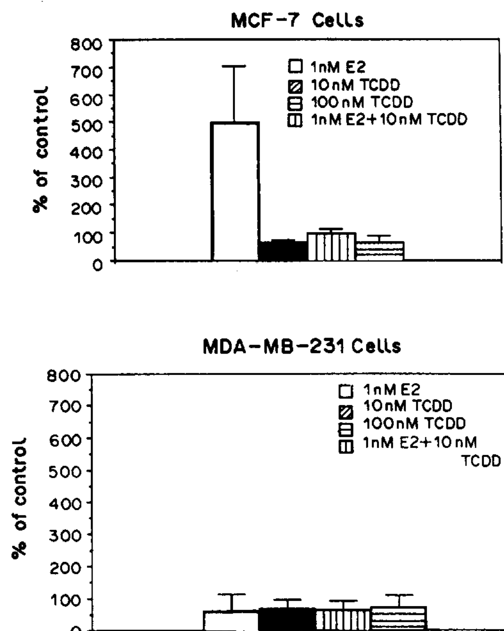

Fig. 3. The effects of 1 nM 17β-estradiol, 10 and 100 nM TCDD and 1 nM 17β-estradiol plus 10 nM TCDD on levels of the 52-kDa protein secreted by MCF-7 (top) and MDA-MB-231 (bottom) cells in culture. The cells were grown and treated with the chemicals as described in the Experimental section and labeling was achieved using [^{35}S]methionine. The levels of radiolabeled 52-kDa protein relative to the control (untreated cells) were determined by fluorography and densitometry. Each experiment was carried out in triplicate and the results are expressed as means ± SD. Treatment with 1 nM 17β-estradiol significantly increased 52-kDa protein levels ($P < 0.01$) and treatment of the cells with TCDD (10 or 100 nM) or TCDD (10 nM) plus 17β-estradiol (1 nM) all significantly decreased levels of excreted 52-kDa protein ($P < 0.01$). In contrast, the levels of the 52-kDa protein secreted by the treated MDA-MB-231 cells were not significantly different ($P < 0.01$) than observed for the solvent control cells.

MCF-7 cells. Comparable results were observed for the ng/DNA per flask (data not shown).

Preliminary time-course experiments indicated that the maximum levels of the 17β-estradiol-stimulated secretion of the 52-kDa protein occurred within 48–96 h after treatment of the cells with 1 nM concentrations of the hormone. Figures 3–5 summarize the relative levels of incorporation of [^{35}S]methionine into the 52-, 34- and 160-kDa proteins secreted by MCF-7 and MDA-MB-231 cells treated with 1 nM 17β-estradiol, 10 nM TCDD, 100 nM TCDD and 10 nM TCDD plus 1 nM 17β-estradiol. In MCF-7 cells treated with 17β-estradiol (1 nM) the levels of the 34-, 52- and 160-kDa proteins secreted into the medium were 372 ± 150, 497 ± 206 and 381 ± 143% of the corresponding levels secreted in control (untreated) cells; the levels of these secreted proteins were significantly decreased in MCF-7 cells treated with 10 nM TCDD (42.3 ± 7.39, 63.7 ± 9.28 and 52.9 ± 19.3%, respectively), 100 nM TCDD (64.0 ± 18.1, 98.2 ± 14.5 and 71.2 ± 18.2%, respectively) and 17β-estradiol (1 nM) plus TCDD (10 nM) (43.8 ± 25.4, 66.3 ± 20.9 and 76.6 ± 48.3%, respectively). In MDA-MB-231 cells with few exceptions, the levels of the 34-, 52- and 160-kDa proteins were not significantly different from the values observed in the control cells after treatment with 17β-estradiol (146 ± 36.5, 61.2 ± 50.6 and 94.7 ± 16.2%, respectively), 10 nM TCDD (136 ± 38.3, 67.1 ± 26.7 and 86.4 ± 12.0%, respectively), 100 nM TCDD (148 ± 50.9, 63.2 ± 29.5 and 120.7 ± 43.0%, respectively) and 17β-estradiol (1 nM) plus 10 nM TCDD (128 ± 56.5, 69.2 ± 41.0 and 113 ± 5.50%, respectively).

Table 1 summarizes the dose-dependent effects of TCDD on the secretion of the 34-, 52- and 160-kDa proteins in MCF-7 cells in the presence or absence of 17β-estradiol. The data indicate that TCDD causes a concentration-dependent inhibition of the estrogen-induced

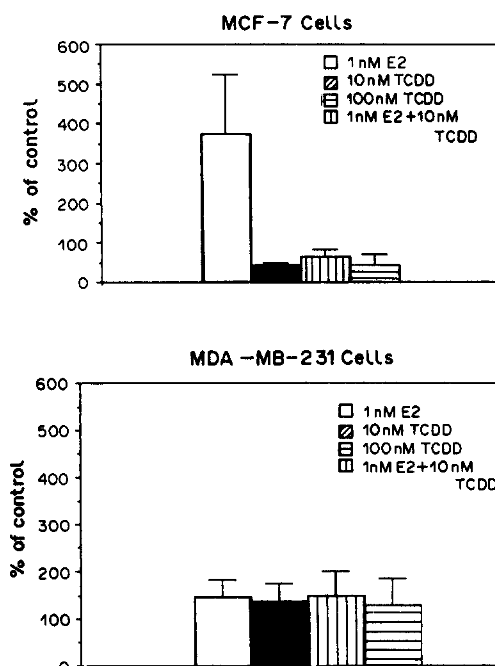

Fig. 4. The effects of 1 nM 17β-estradiol, 10 and 100 nM TCDD and 1 nM 17β-estradiol plus 10 nM TCDD on levels of the 34-kDa protein secreted by MCF-7 (top) and MDA-MB-231 (bottom) cells in culture. The experimental protocols were identical to those described in Fig. 3. The levels of the 34-kDa protein were significantly ($P < 0.01$) greater in cells treated with 17β-estradiol and significantly lower ($P < 0.01$) in cells treated with 10 or 100 nM TCDD and 10 nM TCDD plus 17β-estradiol compared to levels in the solvent control cells. The levels of the secreted 34-kDa protein in the MDA-MB-231 cells were not significantly different in the treated or control cells.

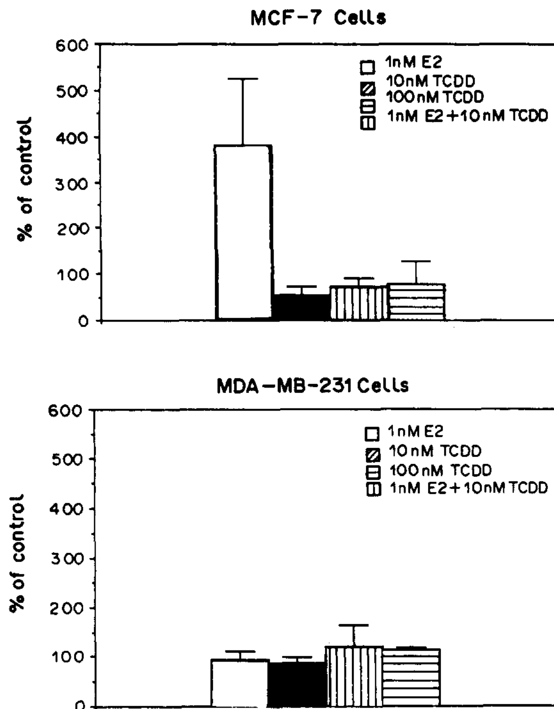

Fig. 5. The effects of 1 nM 17β-estradiol, 10 and 100 nM TCDD and 17β-estradiol plus 10 nM TCDD on levels of the 160-kDa protein secreted by MCF-7 (top) and MDA-MB-231 (bottom) cells in culture. The experimental protocols were identical to those described in Fig. 3. The levels of the 34-kDa protein were significantly ($P < 0.01$) greater in cells treated with 17β-estradiol and significantly lower ($P < 0.01$) in cells treated with 10 or 100 nM TCDD and 10 nM TCDD plus 17β-estradiol compared to levels in the solvent control cells. The levels of the secreted 160-kDa protein in the MDA-MB-231 cells were not significantly different in the treated or control cells.

secretion of all 3 proteins and significant inhibition was observed at concentrations of 1 pM TCDD. Figure 6 illustrates a representative autoradiograph of the ^{35}S-labeled proteins secreted by MCF-7 cells treated with 17β-estradiol (1 nM), TCDD (10 and 100 nM) and 17β-estradiol plus TCDD.

DISCUSSION

MCF-7 human breast cancer cells are an estrogen-responsive human breast cancer cell line which contain relatively high levels of the estrogen receptor. It has also been shown that treatment of MCF-7 cells with TCDD results in the induction of CYP1A1 gene expression and the associated monooxygenases, aryl hydrocarbon hydroxylase and ethoxyresorufin *O*-deethylase activities [30–33]. Moreover, the Ah receptor has also recently been identified in this cell line [32, 33]. The results from this study complement previous reports by Gierthy and co-workers [30, 31] and confirms that TCDD inhibits the growth of MCF-7 cells in culture in both a time- and concentration-dependent manner (Figs 1 and 2). These results are comparable to the growth inhibitory effects of non-steroidal antiestrogens in MCF-7 cells [23, 24]. In contrast, TCDD does not significantly alter the growth characteristics of MDA-MB-231 cells (Fig. 1) and these results correlate with the failure of these cells to respond to the effects of TCDD as an inducer of cytochrome *P*-4501A1 and related monooxygenases [32, 33]. The failure of these cells to respond to TCDD is somewhat paradoxical since the cytosolic and nuclear Ah receptor have been characterized in this cell line [33]. The factor(s) responsible for the Ah non-responsiveness of MDA-MB-231 cells are currently being investigated in this laboratory.

The estrogen-induced secretion of the 52-kDa protein, procathepsin D, in MCF-7 cells has been extensively characterized [1, 11–14, 17, 18, 23]. Treatment of MCF-7 cells with ^{35}S-labeled methionine has been utilized to determine the effects of estrogens and antiestrogens and their combination on the secretion of the 52-kDa protein. This technique

Table 1. Concentration-dependent effects of TCDD on the 17β-estradiol-induced secretion of the 34-, 52- and 160-kDa proteins in MCF-7 cells

Treatment (conc., nM)[a]	Concentration of the ^{35}S-labeled secreted proteins as a per cent of the values for control cells		
	160-kDa	52-kDa	34-kDa
17β-estradiol	371 ± 27.1	225 ± 27.8	282 ± 17.6
TCDD (0.1)	92.8 ± 5.16	103 ± 4.35	94.5 ± 13.3
TCDD (0.01)	90.4 ± 17.2	93.1 ± 15.5	85.7 ± 11.9
TCDD (0.001)	94.3 ± 3.00	87.7 ± 4.89	95.2 ± 12.8
TCDD (0.0001)	97.4 ± 2.73	103 ± 6.63	86.5 ± 8.56
17β-estradiol + TCDD (0.0001)	321 ± 29.0	164 ± 20.3	255 ± 37.8
17β-estradiol + TCDD (0.001)	167 ± 31.3[b]	126 ± 4.62[b]	151 ± 20.7[b]
17β-estradiol + TCDD (0.01)	127 ± 21.0[b]	103 ± 4.39[b]	94.2 ± 24.0[b]
17β-estradiol + TCDD (0.1)	94.3 ± 5.23[b]	94.4 ± 3.80[b]	94.9 ± 11.4[b]

[a]The concentration of 17β-estradiol was 1 nM in all experiments. [b]Significantly lower ($P < 0.01$) than cells treated with 17β-estradiol alone.

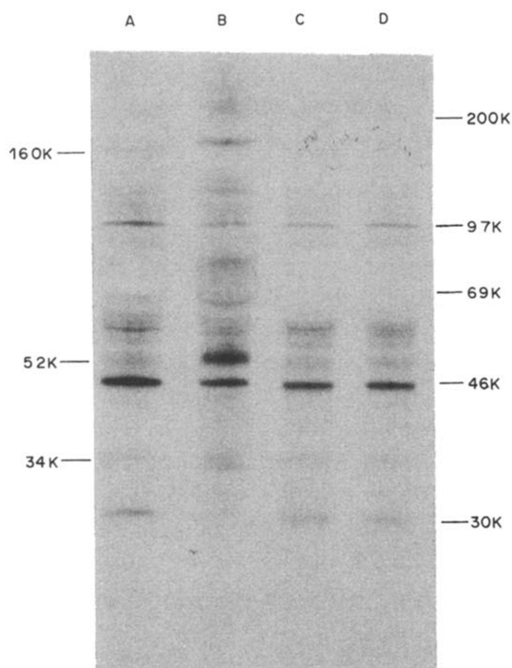

Fig. 6. Autoradiographic analysis of the 34-, 52- and 160-kDa proteins treated with 10 nM TCDD plus 17β-estradiol (lane A), 17β-estradiol (lane B), 10 nM TCDD (lane C) and 100 nM TCDD (lane D).

can also be used to investigate the secretion of other estrogen-induced proteins including the 34-kDa protein, a processed form of procathepsin D [17] and the 160-kDa protein [19]. The results summarized in Figs 3–6 demonstrate that TCDD (10 nM) significantly decreased the 17β-estradiol-induced secretion of the 34-, 52- and 160-kDa proteins in the MCF-7 cells. Moreover, the inhibitory effects of TCDD were also observed in a concentration-dependent fashion as summarized in Table 1. The results obtained in this study for TCDD were comparable to those observed in MCF-7 cells for the non-steroidal antiestrogen, tamoxifen [19].

Figures 3–5 summarize a parallel set of experiments on the effects 17β-estradiol, TCDD and their combination on the levels of the 52-kDa protein secreted in the estrogen non-responsive MDA-MB-231 cell line. The levels of 52-kDa protein in cells treated with 1 nM 17β-estradiol, 10 nM TCDD, 100 nM TCDD and 10 nM TCDD plus 1 nM 17β-estradiol were 61.2, 67.1, 63.2 and 69.2% of the values observed for the control (untreated) cells. However, analysis of the results showed that there were no significant differences between the levels of the 52-kDa protein secreted in all of the treatment groups and in the cells treated with the vehicle control (DMSO). Moreover, comparable results were also observed for the 34- and 160-kDa proteins and these data demonstrate that the interactive effects of 17β-estradiol and TCDD in MDA-MB-231 cells were not significant with respect to the secretion of these proteins. These results are comparable to the effects of TCDD, 17β-estradiol and TCDD plus 17β-estradiol on cell growth (Fig. 1) and confirm the Ah non-responsiveness of this cell line. The results of this study demonstrate that TCDD, like tamoxifen [19] acts as a full estrogen antagonist in MCF-7 cells. TCDD does not exhibit agonist or antagonist activity in the Ah non-responsive MDA-MB-231 cell line, however Rochefort and co-workers have reported that tamoxifen increased the secretion of the 52-kDa protein in the estrogen-insensitive R27 and RTx6 human breast cancer cell lines [19].

The results reported in this study show that TCDD caused a concentration-dependent decrease in the secretion of the 17β-estradiol-induced 34-, 52- and 160-kDa proteins in MCF-7 cells (Table 1) and TCDD also suppressed cell growth and proliferation. Results from previous studies suggest that there are at least two possible mechanisms of action for TCDD as an antiestrogen. The structure-dependent activities of TCDD [26–29] are consistent with a direct role for the Ah receptor which presumably elicits antiestrogenic effects through altered gene transcription [34]. Gierthy and co-workers [35] have reported that the TCDD-mediated induction of aryl hydrocarbon hydroxylase activity is paralled by induced 17β-estradiol hydroxylase activities. These data suggest that TCDD may also act as an antiestrogen by increasing the cellular oxidative metabolism of 17β-estradiol thereby decreasing constitutive levels of the hormone. It has previously been shown that TCDD does not significantly induce aryl hydrocarbon hydroxylase activity in MCF-7 cells at concentrations of 0.01 nM and lower [33]. However, the results summarized in Table 1 demonstrate that TCDD significantly inhibited the 17β-estradiol-induced secretion of the 34-, 52- and 160-kDa proteins in MCF-7 cells at concentrations of 0.01 and 0.001 nM. These data not only illustrate the remarkable potency of TCDD as an antiestrogen but also suggest that these effects are not due to TCDD-induced metabolism of 17β-estradiol. This does not necessarily exclude this pathway as a factor for other antiestrogenic effects of TCDD which may be observed at higher concentrations (i.e > 0.01 nM).

These results coupled with data from previous studies [25–31] confirm the broad spectrum antiestrogenic activity of TCDD in estrogen-responsive cells and target tissues. Moreover, the differential responsiveness of the MCF-7 (Ah responsive) and MDA-MB-231 (Ah nonresponsive) cells to the antiestrogenic effects of TCDD and the concentration-dependent activities (Table 1) are consistent with a role for the Ah receptor in mediating this process. The identity of TCDD-induced factors which may be involved in the antiestrogenic action of this compound are currently being investigated.

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REFERENCES

1. Rochefort H., Chalbos D., Capony F., Garcia M., Veith F., Vignon F. and Westley B.: Effect of estrogen in breast cancer cells in culture: released proteins and control of cell proliferation. In *Hormones and Cancer, Progress in Clinical and Biological Research* (Edited by E. Gurpide, R. Calandra, C. Levy and R. J. Soto). Liss, New York (1984) pp. 37-51.
2. Lippman M. E., Huff K. K., Jakesz R., Hecht T., Kasid A., Bates S. and Dickson R. B.: Estrogens regulate production of specific growth factors in hormone-dependent human breast cancer. In *Estrogen/Antiestrogen Action and Breast Cancer Therapy* (Edited by C. V. Jordan). The University of Wisconsin Press, Madison (1986) pp. 237-247.
3. Miller M. A., Sheen Y. Y., Mullick A. and Katzenellenbogen B. S.: Antiestrogen binding to estrogen receptors and additional antiestrogen binding sites in human breast cancer cells. In *Estrogen/Antiestrogen Action and Breast Cancer Therapy* (Edited by C. V. Jordan). The University of Wisconsin Press, Madison (1986) pp. 127–148.
4. Dickson R. B. and Lippman M. E.: Estrogenic regulation of growth and polypeptide growth-factor secretion in human breast carcinoma. *Endocr. Rev.* **8** (1987) 29–43.
5. Dickson R. B. and Lippman M. E.: Hormonal control of human breast cancer cells. *Cancer Surv.* **5** (1986) 617–624.
6. Lippman M., Bolan G. and Huff K.: The effects of estrogens and antiestrogens of hormone-responsive human breast cancer in long-term tissue culture. *Cancer Res.* **36** (1976) 4595–4601.
7. Katzenellenbogen B. S., Kendra K. L., Norman M. J. and Berthois Y.: Proliferation, hormonal responsiveness and estrogen receptor content of MCF-7 human breast cancer cells grown in the short-term and long-term absence of estrogens. *Cancer Res.* **47** (1987) 4355–4360.
8. Bronzert D. A., Monaco M. E., Pinkus L., Aitken S. C. and Lippman M. E.: Purification and properties of estrogen-responsive cytoplasmic thymidine kinase from human breast cancer. *Cancer Res.* **41** (1981) 604–610.
9. Cowan K., Levine R., Aitken S., Goldsmith M., Douglass E., Clendenin N., Niehaus A. and Lippman M. E.: Dihydrofolate reductase gene amplification and possible rearrangement in estrogen-responsive methotrexate-resistant human breast cancer cells. *J. Biol. Chem.* **257** (1982) 15,079–15,086.
10. Thomas M., Monet J. D., Brami M., Dautigany N., Assaily J., Ulmann A. and Bader C. A.: Comparative effects of 17β-estradiol, progestin, R5020, tamoxifen and RU38486 on lactate-dehyrogenase activity in MCF-7 human breast cancer cells. *J. Steroid Biochem.* **32** (1989) 271–277.
11. Westley B. and Rochefort H.: A secreted glycoprotein induced by estrogen in human breast cancer cell lines. *Cell* **20** (1980) 353–362.
12. Vignon F., DeRocq D., Chambon P. and Rochefort H.: Estrogen-induced proteins secreted by the MCF-7 human breast cancer cells stimulate their proliferation. *C.R. Acad. Sci.* **296** (1983) 151–156.
13. Dickson R. B., Huff K. K., Spencer E. M. and Lippman M. E.: Induction of epidermal growth factor-related polypeptides by 17β-estradiol in MCF-7 human breast cancer cells. *Endocrinology* **118** (1986) 138–142.
14. Morisset M., Capony F. and Rochefort H.: Processing and estrogen regulation of the 52-kDa protein inside MCF-7 breast cancer cells. *Endocrinology* **119** (1986) 2773–2782.
15. Shyamala V. and Dickerman H. W.: Two types of plasminogen activators secreted by MCF-7 cells. *Biochem. Biophys. Res. Commun.* **138** (1982) 102–109.
16. Ryan T. J., Seeger J. I., Kumar S. A. and Dickerman H. W.: Estradiol preferentially enhances extracellular tissue plasminogen activators of MCF-7 breast cancer cells. *J. Biol. Chem.* **259** (1984) 14,324–14,327.
17. Morisset M., Capony F. and Rochefort H.: The 52-kDa estrogen-induced protein secreted by MCF-7 cells in a lysosomal acidic protease. *Biochem. Biophys. Res. Commun.* **138** (1986) 102–109.
18. Briozzo P., Morisset M., Capony F., Rougeot C. and Rochefort H.: *In vitro* degradation of extracellular matrix with M_r 52,000 cathepsin D secreted by breast cancer cells. *Cancer Res.* **48** (1988) 3688–3692.
19. Rochefort H., Vignon F., Bardon S., May F. and Westley B.: Different efficacy of antiestrogens on estrogen-regulated proteins in human breast cancer cell lines. In *Estrogen/Antiestrogen Action and Breast Cancer Therapy* (Edited by C. V. Jordan). The University of Wisconsin Press, Madison (1986) pp. 249–263.
20. Spyratos F., Brouillet J.-P., DeFrenne A., Hacene K., Rouesse J., Maudelande T., Brunet M., Andrieu C., Desplaces A. and Rochefort H.: Cathepsin D: an independent prognostic factor for metastasis of breast cancer. *Lancet* **November 11** (1989) 115–118.
21. Tandon A. K., Clark G. M., Chamness G. C., Chirgwin J. M. and McGuire W. L.: Cathepsin D and prognosis in breast cancer. *New Engl. J. Med.* **322** (1990) 297–302.
22. Aitken S. C. and Lippman M. E.: Effect of estrogens and antiestrogens on growth-regulatory enzymes in human breast cancer-cells in tissue culture. *Cancer Res.* **45** (1985) 1611–1620.
23. Westley B., May F. E. B., Brown M. C., Krust A., Chambon P., Lippman M. E. and Rochefort H.: Effects of antiestrogens on the estrogen-regulated PS2 RNA and the 52-kDa and 160-kDa proteins in MCF-7 cells and two tamoxifen resistant sublines. *J. Biol. Chem.* **259** (1984) 10,030–10,035.
24. Thompson E. W., Reich R., Shima T. B., Albini A., Graf J., Martin G. R., Dickson R. B. and Lippman M. E.: Differential regulation of growth and invasiveness of MCF-7 breast cancer cells by antiestrogens. *Cancer Res.* **48** (1988) 6764–6768.
25. Gallo M. A., Hesse E. J., MacDonald G. J. and Umbreit T. H.: Interactive effects of estradiol and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on hepatic cytochrome *P*-450 and mouse uterus. *Toxic. Lett.* **32** (1986) 123–132.

26. Romkes M., Piskorska-Pliszczynska J. and Safe S.: Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on hepatic and uterine estrogen receptor levels in rats. *Toxic. Appl. Pharmac.* **87** (1987) 306–314.
27. Romkes M. and Safe S.: Comparative activities of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and progesterone as antiestrogens in female rat uterus. *Toxic. Appl. Pharmac.* **92** (1988) 368–380.
28. Astroff B. and Safe S.: Comparative antiestrogenic activities of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 6-methyl-1,3,8-trichlorodibenzofuran in the female rat. *Toxic. Appl. Pharmac.* **95** (1988) 435–443.
29. Astroff B. and Safe S.: 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin as an antiestrogen: effect on rat uterine peroxidase activity. *Biochem. Pharmac.* **39** (1990) 485–488.
30. Gierthy J. F., Lincoln D. W., Gillespie M. B., Seeger J. I., Martinez H. L., Dickerman H. W. and Kumar S. A.: Suppression of estrogen-regulated extracellular plasminogen activator activity of MCF-7 cells by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Cancer Res.* **47** (1987) 6198–6203.
31. Gierthy J. F. and Lincoln D. W.: Inhibition of post-confluent focus production in cultures of MCF-7 breast cancer cells by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Breast Cancer Res. Treat.* **12** (1988) 227–233.
32. Vickers P. J., Dufresne M. J. and Cowan K. H.: Relation between cytochrome *P*4541A1 expression and estrogen receptor content of human breast cancer cells. *Molec. Endocr.* **3** (1989) 157–164.
33. Harris M., Piskorska-Pliszczynska J., Zacharewski T., Romkes M. and Safe S.: Structure-dependent induction of aryl hydrocarbon hydroxylase in human breast cancer cell lines and characterization of the Ah receptor. *Cancer Res.* **49** (1989) 4531–4535.
34. Whitlock J. P.: The regulation of gene expression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Pharmac. Rev.* **39** (1987) 147–161.
35. Gierthy J. F., Lincoln D. W., Kampcik S. J., Dickerman H. W., Bradlow H. L., Niwa T. and Swaneck G. E.: Enhancement of 2- and 16α-estradiol hydroxylation in MCF-7 human breast cancer cells by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Biochem. Biophys. Res. Commun.* **157** (1988) 515–520.