MECHANISM OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) DOWNREGULATION OF THE ESTROGEN RECEPTOR IN THE MCF-7 BREAST CANCER CELL LINE

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Abstract

TCDD elicits a diverse spectrum of biochemical and toxic responses such as the induction of phase I and phase II drug-metabolizing enzymes. The molecular mechanism for TCDD-induced responses is mediated through internal binding to a cytosolic protein, the aryl hydrocarbon (Ah) receptor. TCDD exhibits antiestrogenic activity in estrogen-responsive organs and cell lines. In this study, treatment of MCF-7 cells with 1 nM TCDD caused a time-dependent decrease of estrogen receptor (ER) levels as analyzed by velocity sedimentation analysis. ER levels decreased to 63% of the control values within 3 hr after treatment and was further decreased after 24 hr. Gel mobility shift analysis demonstrated that TCDD inhibited the estrogen responsive element (ERE)-ER binding in a time-dependent manner. The inhibition of ER-ERE binding was observed within 1 hr after treatment of cells with TCDD and was maximal after 12 hr. Co-treatment of cells with α-naphthoflavone (αNF), an Ah receptor antagonist, inhibited TCDD-mediated downregulation of both ER levels and ERE binding. Co-treatment with cycloheximide did not restore these values. This data indicates that TCDD-induced downregulation of ER levels and ER-ERE binding is Ah receptor-mediated.

Introduction

MCF-7 human breast cancer cells are estrogen responsive and express relatively high levels of the estrogen receptor (ER). A number of human cancers including leukemia, prostate, endometrial and breast carcinoma are known to be regulated by endocrine factors. Beatson first observed that ovarian secretion of estrogens was linked to breast carcinoma and endocrine ablation was among the first successful technique used for the treatment of breast cancer.[1] Human breast cancer cell lines have been extensively used to investigate the cellular and molecular mechanisms associated with the growth of these cells, and the

development of strategies which can be used for the clinical treatment of breast cancer. Several studies have demonstrated that breast cancer cell lines can undergo changes during prolonged exposure to compounds such as antiestrogens and this can lead to major alterations in the phenotype of the resultant breast cancer cell lines. Aromatic hydrocarbons, typified by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), has been identified as a new class of antiestrogens that act through the aryl hydrocarbon (Ah) receptor. It has been demonstrated that TCDD exhibits a broad spectrum of antiestrogenic activities in human breast cancer cell lines.

TCDD exhibits antiestrogenic activity in estrogen-responsive organs and cell lines. Gierthy and coworkers reported the antiestrogenic effects of TCDD in human breast cancer cells [2-3]. Their results showed that TCDD suppressed the E2-induced secretion of tissue plasminogen activator activity, cell proliferation and postconfluent focus production in MCF-7 cells. Research in this laboratory has also focused on the antiestrogenic effects of TCDD in human breast cancer and rodent cell lines [4-5]. In cell proliferation studies, TCDD inhibited the growth of ER-positive MCF-7 cells, but did not alter the growth of ER-negative MDA-MB-231 cells. TCDD also inhibited the E2-induced secretion of the 32-, 54- and 160-kDa proteins in MCF-7 cells. Thus, TCDD is a broad spectrum antiestrogen and there appears to be an interaction between the AhR- and ER-dependent endocrine systems.

This study will investigate the molecular mechanisms of TCDD-mediated antiestrogenicity in MCF-7 human breast cancer cells and delineate the nature the role of the Ah receptor in this process.

Materials and Methods

Treatment of 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 plus 10 ml antibiotic-antimycotic solution (Sigma) in an air:carbon dioxide (95:5) atmosphere at 37°C. Cells were grown in DMEF/12 medium without phenol red for 2 days before harvesting.

Sucrose density gradient analysis and nuclear extraction: Sucrose density analysis nuclear extraction was performed according to the described procedure[6].

Gel shift assay: DNA binding was measured using a gel shift assay with a complementary pair of oligonucleotides containing the sequence 5'-GTCCAAAGTCAGGTCACAGTGACCTGATCAAAGTT-3' [7].

Results and Discussion

The major objective of this study was to investigate the mechanisms associated with the TCDD-mediated decrease in nuclear ER levels in MCF-7 cells. Initial studies focused on the time-dependent effects of 1 nM TCDD on both nuclear ER binding and formation of the retarded nuclear ER-ERE complex.

In MCF-7 cells treated with 1 nM [^{3}H]17β-estradiol (for 1 hr) and 1 nM TCDD for 1 to 24 hr, there was an initial increase in nuclear ER binding whereas significantly decreased ER binding was observed 3 to 24 hr after treatment with TCDD (Table I). In comparable binding studies, it was shown that cycloheximide did not affect the TCDD-induced decrease in ER binding and this suggested that protein synthesis was not involved in this response. In contrast, α-naphthoflavone, an Ah receptor antagonist partially inhibited the decrease in ER binding, thus confirming the role of the Ah receptor in mediating this effect (Table II). In parallel experiments using MCF-7 cells cultured in non-stripped fetal calf serum, the time dpendent effects of TCDD on the binding of nuclear extracts to a consensus [^{32}P] ERE was determind using a gel mobility shift assay. The results show that within 1 hr after treatment with TCDD, there was a significant decrease in levels of the ER-ERE retarded band and this decrease persisted for up to 24 hr (Table I). Moreover, the effects of cycloheximide and α-naphthoflavone on the decrease in ER binding caused by TCDD were also observed for the TCDD-mediated decrease in ER-ERE binding as determing by gel mobility shift assays (Table II). Within 1 or 3 hr after treatment with 1 nM TCDD, there was a significant decrease in nuclear ER levels determined by either gel retardation or velocity sedimentation analysis; the effects were not blocked by cycloheximide but were significantly inhibited by α-naphthoflavone. This data indicates that TCDD-induced downregulation of ER levels and ER-ERE binding is Ah receptor-mediated and acts possibly at the translational or posttranslational level.

Table I. Time-dependent effects of TCDD on the downregulation of nuclear ER levels and the intensity of the ER-ERE retarded band in the human breast cancer MCF-7 cell line.

Treatment (time,hr)	ER-[3H]Estradiol Complex[1] (fmol/mg protein)	ER-ERE Complex[2] (CPM)
Control	468±13.7	198±4
TCDD (1)	570±11.6*	149±7*
TCDD (3)	307±2.5*	117±8*
TCDD (6)	279±0.5*	100±5*
TCDD (12)	264±6.1*	71±1*
TCDD (24)	335±5.1*	74±1*

* Significantly different ($p<0.01$) than control.
[1]Determined by velocity sedimentation analysis.
[2]Determined by gel mobility shift assay.

Table II. Effects of α-naphthoflavone or cycloheximide on the TCDD-mediated decrease of nuclear ER levels and ER-ERE retarded band.

Treatment (conc.)	ER-[^{3}H] Estradiol Complex[1] (fmol/mg protein)	ER-ERE Complex[2] (CPM)
[^{3}H]17β-Estradiol (1 nM)	567±15	38.8±1.1
TCDD (1 nM)	375±5.7[a]	26.5±2.1[a]
α-Naphthoflavone (1 mM)	542±23	35.5±4.5
Cycloheximide (10 mM)	528±13	29.4±2.4
TCDD + α-Naphthoflavone	437±7[a]	39.7±2.4[a]
TCDD + Cycloheximide	314±7.9[a]	21.5±3.8[a]

[a] Significantly different ($p<0.01$) than control

[1] Determined by velocity sedimentation analysis (6 hr)

[2] Determined by gel mobility shift assay (3 hr)

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