INHIBITION OF ESTROGEN-INDUCED HEAT SHOCK PROTEIN 27 (HSP 27) GENE EXPRESSION BY 2,3,7,8-TETRACHLORODIBENZO-*p* -DIOXIN (TCDD) IN MCF-7 HUMAN BREAST CANCER CELLS

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1. Abstract

Treatment of MCF-7 cells with 10 nM TCDD for 2 or 24 hr with 10 nM 17β-estradiol (E2) resulted in approximately 2-fold induction of Hsp 27 mRNA levels. Ten nM TCDD alone did not affect Hsp 27 mRNA levels after 2 or 24 hr however in MCF-7 cells cotreated with TCDD plus E2, the E2-induced Hsp 27 mRNA levels were significantly inhibited after treatment with TCDD for 2 or 24 hr. These results further extend the antiestrogenic activity of TCDD in MCF-7 cells and illustrate the interaction between the aryl hydrocarbon (Ah) and estrogen receptor (ER) mediated signal transduction pathways.

2. Introduction

E2 induces proliferation of estrogen receptor (ER)-positive breast cancer cells in culture and plays a role in the development and growth of human mammary cancer. E2 also induces a gene battery in ER-positive breast cancer cells and this includes induction of the progestrone receptor, Hsp 27, cathepsin D, transforming growth factor α, pS2, insulin-like growth factor binding protein 4 and several protooncogenes mRNAs.[1,2,3] Levels of several of these gene products have been determined in mammary tumors and some are used as prognostic indicators for women with breast cancer and for determining the type of treatment regimens.[4] Several studies have reported that Hsp 27, a low molecular weight member of the Hsp family, is induced by E2 in MCF-7 human breast cancer cells.[5] The biological function of Hsp 27 has not been defined, however there are studies which suggest that this protein may play a role in cellular thermotolerance, act as a protein chaperone or function as a signaling molecule in diverse signal transduction pathways. [6,7,8] Levels of Hsp 27 in mammary tumor have been determined and results indicate that approximately 90% of mammary tumors which express Hsp 27 protein are also ER-positive. The potential utility of Hsp 27 as a clinical marker in breast cancer has not been resolved and will require further comparisons with other diagnostic markers. [9]

Several reports have characterized TCDD as an antiestrogen in the rodent uterus and ER-positive human breast cancer cell lines in culture and the results of these studies show that TCDD also inhibits E2-induced Hsp 27 mRNA levels in MCF-7 human breast cancer cell lines.

3. Material and Methods

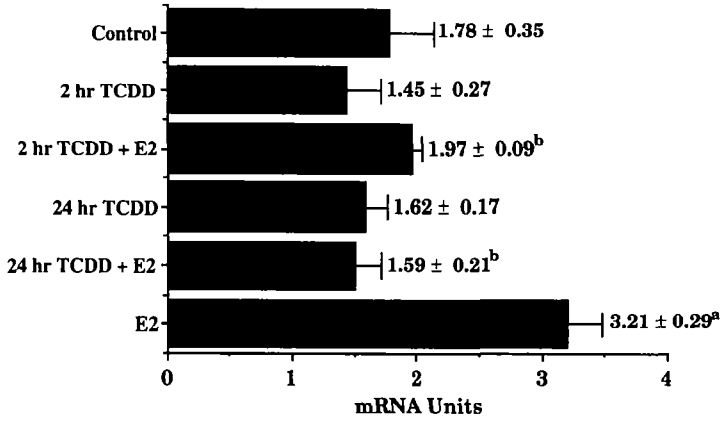
Treatment of cells: The human breast cancer MCF-7 cell line was obtained from the American Type Culture Collection (ATCC, Rockville, MD). Cells were maintained in DMEF/12 medium with phenol red and supplemented with 5% fetal bovine serum plus 10 ml antibiotic-antimyotic solution (Sigma) in an air:carbon dioxide (95:5) atmosphere at 37°C. Cells were grown in DMEF/12 medium without phenol red and 5% stripped fetal bovine serum two days before dosing.

Northern Analysis: The plasmid pUCHS208 containing the HSP 27 cDNA was purchased from StressGen (Victoria, B.C., Canada) The plasmid which contains the β-tubulin gene was obtained from ATCC. RNA was extracted from the cells treated with DMSO (control), TCDD, 17β-estradiol or combinations by using the acidic guanidinium thiocyanate procedure followed by separation on a 1.2% agarose gel electrophoresis and then transferred to a nylon membrane. The membrane was then exposed to UV light for 5 min to crosslink the RNA to the membrane and baked at 80°C for 2 hr. The membrane prepared in this manner was prehybridized in a solution containing 0.1% BSA, 0.1% Ficoll, 0.1& polyvinylpyrollidone, 10% dextran sulfate, 1% SDS and 5 X SSPE (0.75 M NaCl, 50 nM NaH_2PO_4, 5 mM EDTA) for 18 to 24 hr at 65°C and hybridized in the same buffer for 24 hr with the ^{32}P-labeled DNA probe (10^6 cpm/ml). The cDNA probes were labeled with [α-^{32}P]CTP using the random primed DNA labeling kit (Boehringer-Mannheim, Indianapolis, IN, USA). The resulting blots were quantitated using a Betagen Betascope 603 blot analyzer and visualized by autoradiography. The Hsp 27 mRNA levels were standardized against β-tubulin mRNA.

4. Results and Discussion

The data summarized in Figure I illustrate the effects of 10 nM E2, 10 nM TCDD and their combination on Hsp 27 mRNA levels in MCF-7 cells. E2 causes a rapid induction of Hsp 27 mRNA levels (within 2 hr) which remain elevated for up to 24 hr. In contrast, 10 nM TCDD does not significantly induce Hsp 27 mRNA levels after exposure of MCF-7 cells for 2 or 24 hr. In cells co-treated with TCDD plus E2, TCDD significantly inhibited E2-induced Hsp 27 mRNA levels after 2 or 24 hr. Previous studies in several laboratories have demonstrated that TCDD inhibited E2-induced proliferation of MCF-7 cells and a number of E2-induced responses including secretion of the 52-, 34-, and 160-kDa proteins, postconfluent focus production and PR gene expression.[3,10] Recent studies in this laboratory have demonstrated that TCDD inhibits E2-induced cathepsin D gene expression by direct interaction of the nuclear Ah receptor complex with strategically located genomic dioxin responsive elements (DRE's) in the 5'-promoter region of the gene.[11] It has also been proposed by Gierthy and coworkers that the antiestrogenic effects of TCDD are primarily related to induced P450 which results in increased metabolism of E2 and cellular depletion

of the hormone.[12] In this study, the early antiestrogenic effects of TCDD (within 2hr) indicate that E2 metabolism does not play a role in the inhibition of E2-induced Hsp 27 mRNA levels since the P450 induction response is not observed until 3-6 hr after treatment with TCDD. Current studies in this laboratory are focused on the molecular mechanisms associated with inhibition of estrogen-induced Hsp 27 gene expression.



[a] Significantly different ($P < 0.05$) from control
[b] Significantly different ($P < 0.01$) from E2

Figure I. Downregulation of Estrogen Induced Hsp 27 mRNA by TCDD

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5. References

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