ANALYSIS OF THE ANTI-ESTROGENIC EFFECT OF TCDD IN HUMAN OVARIAN CARCINOMA (BG-1) CELLS

Jane M. Rogers and Michael S. Denison

Department of Environmental Toxicology, University of California, Davis, CA 95616 USA

Introduction

Concerns have been raised over the last decade regarding the prevalence in the environment of compounds that are able to disrupt the estrogen homeostasis of an organism. Although effects on human health remain controversial, the reproductive success of several wildlife populations has been detrimentally affected by such compounds[1,2]. Often, these compounds act as estrogen agonists by interacting directly with the estrogen receptor (ER). However, the halogenated aromatic hydrocarbon 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), is known to exhibit an array of anti-estrogen effects *in vivo* and in cells in culture, yet the mechanism(s) for this inhibition has not been elucidated. Numerous investigators have focused on determining the mechanism for the anti-estrogenic action of TCDD. Several hypotheses have been proposed, including the increased metabolism and clearance of estradiol by TCDD induced enzymes[3] and down-regulation of ER mRNA and protein[4-6]. It has also been proposed that the aryl hydrocarbon receptor (AhR) interacts with an inhibitory dioxin responsive element (iDRE) located adjacent to, or overlapping, the ER binding site in the upstream regions of the cathepsin-D and pS2 genes, thereby blocking ER-DNA binding via steric hinderance[7,8]. However, this mechanism does not seem to be responsible for the TCDD induced repression of other estrogen induced genes [9].

In previous work we have demonstrated the AhR-dependent inhibition of estrogen signaling by TCDD in wild type human ovarian carcinoma (BG-1) cells and in recombinant BG-1 cells ($BG1Luc4E_2$) that have been stably transfected with an estrogen-responsive luciferase reporter gene[10]. We have also examined individual steps in the pathway of estrogen signaling in order to determine those that are affected by TCDD. We have previously demonstrated that pre-treatment of $BG1Luc4E_2$ cells with TCDD failed to increase the inhibitory effect and that 1,000-fold excess estradiol did not eliminate the effect of TCDD[10]. TCDD also decreased ER protein and mRNA levels under standard media conditions. However, growth in estrogen-stripped media (ESM) (which is used to decrease background luciferase levels) decreased ER protein and mRNA levels by about 7-fold without altering either the AhR level or function[10]. This decrease in ER but not AhR results in a significant (~ 7-fold) increase in the AhR/ER ratio in cells grown in ESM compared to those grown in standard media, and yet no change in maximal luciferase induction or the degree of inhibition by TCDD was observed. We also demonstrated that TCDD does not decrease ER protein levels when cells are grown in ESM[10]. Taken together, these results from our previous studies suggest that the TCDD-dependent inhibition of estrogen signaling in $BG1Luc4E_2$ cells is not mediated by a decrease in either estradiol or ER levels. In the present study we have extended this work by examining additional steps in the pathway of estrogen signal transduction in an attempt to determine the mechanism by which TCDD inhibits estrogen signaling in BG-1 and $BG1Luc4E_2$ cells.

Materials and Methods

Luciferase Expression Assay for BG1Luc4E$_2$ Cells. Cells were grown for 6 days in estrogen stripped media (ESM) (phenol red free MEM with 5% dextran-coated charcoal stripped FBS), with daily media changes. Cells were exposed to the indicated chemical for increasing amounts of time, followed by lysis and measurement of luciferase activity as described in detail[11].

Northern Blot Analysis. RNA from treated cells was isolated using the RNeasy Mini Kit from Qiagen. Northern blot analysis was carried out on total RNA (20 μg) as previously described[12]. The resulting blots were visualized by autoradiography and quantified using a Molecular Dynamics PhosphorImager SI, and pS2 values were normalized to those of GAPDH.

Results and Discussion

To determine how rapidly TCDD inhibits estrogen-induced luciferase activity we analyzed the time-course of TCDD inhibition in BG1Luc4E$_2$ cells grown in ESM. In general, TCDD-dependent inhibition occurred between 7 hours and 18 hours after treatment (fig. 1). These results suggest that the effect of TCDD on estrogen-induced luciferase activity was not a direct, primary event, which would have been observed much earlier, but was the result of an indirect, secondary response. To further examine whether the anti-estrogenic action of TCDD in BG1Luc4E$_2$ cells is mediated directly by the AhR or indirectly by a TCDD-induced protein, BG1Luc4E$_2$ cells were grown in standard media and the effect of the protein synthesis inhibitor cyclohexamide (CHX) on the TCDD-dependent inhibition of the endogenous estrogen-dependent pS2 gene expression was examined. TCDD reduced pS2 mRNA levels to 60% - 70% of that of DMSO treated cells, however, in the presence of cyclohexamide, TCDD did not significantly reduce pS2 mRNA levels as compared to the DMSO control (fig. 2). These data strongly support a role for a TCDD-induced protein as being responsible for mediating the inhibition of estrogen signaling in BG-1 cells.

A critical step in the pathway of estrogen signaling is the binding of the ER complex to DNA. To examine the effect of TCDD on ER-DNA binding we first determined whether the AhR could directly bind the estrogen responsive element (ERE) which was responsible for estrogen signaling in our reporter system. Although we observed that reticulocyte lysate expressed TCDD:AhR:Arnt complex could bind to a [^{32}P] DRE oligonucleotide, no TCDD-inducible protein-DNA complex was formed using a [^{32}P]-labelled oligonucleotide containing the ERE from the chicken vitellogenin A2 gene. We also examined the ability of nuclear proteins from control and TCDD treated BG1Luc4E$_2$ cells, grown in standard media, to bind these oligonucleotides. As expected, a small TCDD induced protein-DNA band was formed with the [^{32}P] DRE oligonucleotide, however no TCDD-inducible AhR-DNA complex was formed with the chicken vitellogenin ERE. These results clearly indicate that the AhR does not bind, at least *in vitro*, to the ERE we have used in our system. The ER:ERE complex that formed was unaffected by TCDD treatment, indicating that, *in vitro*, TCDD does not alter binding of the ER to DNA, even though total cellular ER levels are decreased by approximately 40%[10]. We therefore propose a scenario for the inhibition of estrogen signaling by TCDD in BG1Luc4E$_2$ cells which results from a TCDD- and AhR-dependent induction of an as yet, unknown "repressor" protein. Our data suggests that this TCDD-induced protein acts at a step that follows ER/DNA binding and most likely occurs at the transcriptional or post-transcriptional level. A model of the proposed mechanism of the anti-estrogenic action of TCDD in BG1Luc4E$_2$ cells is shown in figure 3.

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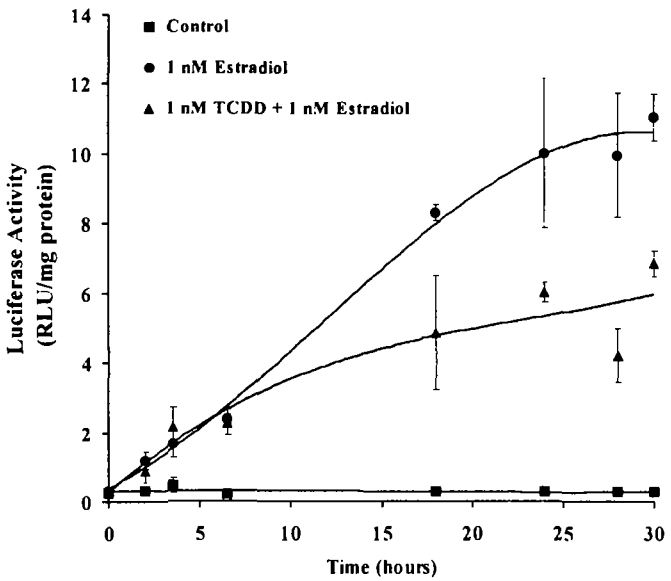


Figure 1. Time course of inhibition of estrogen-induced luciferase activity by TCDD in BG1Luc4E_2 cells. Cells were grown for 6 days in ESM and then treated with the indicated chemical for increasing amounts of time. Luciferase activity was determined as described. Values represent mean ± SD of four determinations.

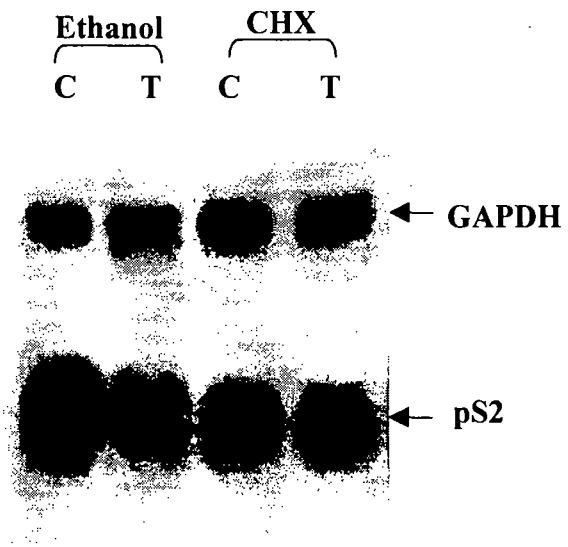
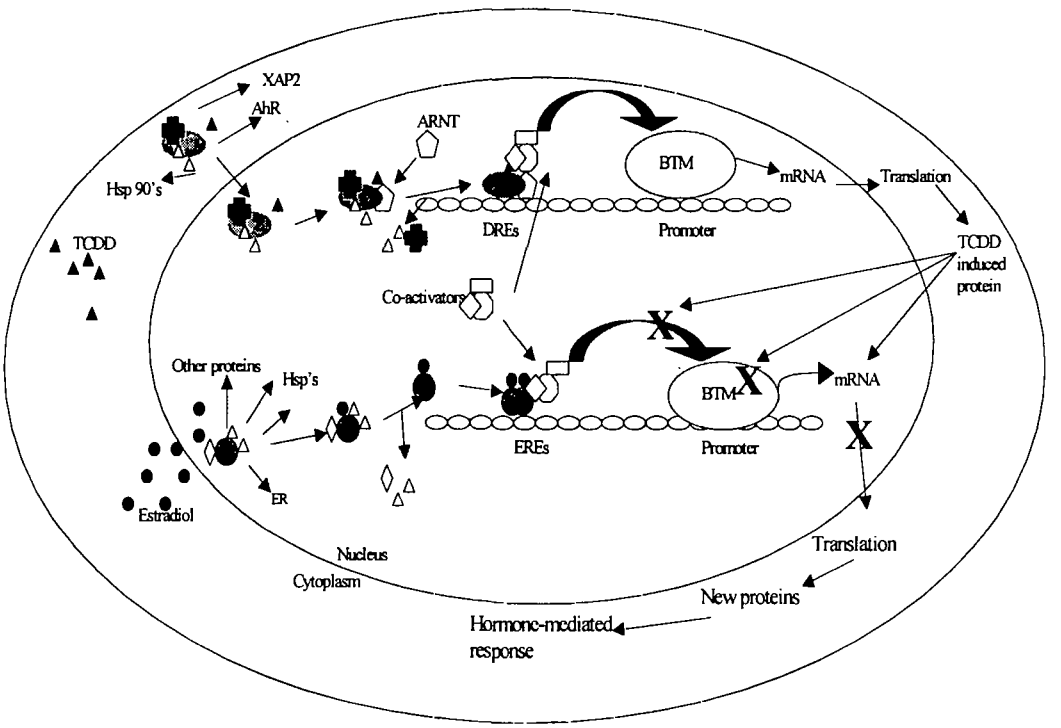


Figure 2. Cyclohexamide blocks TCDD-dependent inhibition of estrogen-induced pS2 mRNA expression in BG1Luc4E$_2$ cells. Cells were grown in standard media and treated for 24 hours with DMSO (C) or 10 nM TCDD (T) with or without 10 μM cyclohexamide (CHX). Isolation of RNA



and Northern analysis was carried out as described in Materials and Methods. This experiment was performed in triplicate with similar results, and a representative blot is shown.

Figure 3. Proposed mechanism for the TCDD-dependent decrease in estrogen signaling in BG1Luc4E$_2$ cells. Abbreviations: BTM (basal transcriptional machinery), Hsp's (heat shock proteins), DREs (dioxin responsive elements), EREs (estrogen responsive elements), AhR (aryl hydrocarbon receptor), ARNT (AhR nuclear translocator), XAP2 (hepatitis B virus X-associated protein 2).

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