2,3,7,8-TETRACHLORODIBENZO-*p* -DIOXIN (TCDD)-MEDIATED INHIBITION OF AN ESTROGEN RESPONSIVE *XENOPUS LAEVIS* VITELLOGENIN A2-CAT CONSTRUCT

K.I.Nodland,[A] O.L.Arellano,[A] S.Safe,[A] G.U.Ryffel,[B] L.Klein-Hitpass[B]
A. Department of Veterinary Physiology and Pharmacology,
Texas A&M University, College Station, TX 77843
B. Institut fur Zellbiologie, Universitatsklinikum Essen, Essen,Germany

ABSTRACT

The plasmid construct pA2(-821/+14)-CAT was derived from the 5' region of the vitellogenin A2 gene of *Xenopus laevis* linked to a chloroamphenicol acetyltransferase (CAT) gene. Vitellogenin A2 is under strict estrogen control and contains a core genomic estrogen response element (ERE) within the 5' (-331/-308) region. This plasmid was transfected into MCF-7 cells which were treated with 1 nM 17β-estradiol (E2), 10 or 100 nM TCDD and TCDD plus E2. Results showed that E2 significantly induced CAT activity, TCDD did not alter this activity, and in cells cotreated with TCDD plus E2, TCDD significantly decreased the E2-induced response. Replacement of the promoter with the heterologous *Herpes* virus thymidine kinase (tk) promoter did not alter the TCDD mediated suppression of E2 inducibility. The results of transfections into wild type Hepa1c1c7 and class II mutant cells with Ah receptor analogs and α-naphthoflavone, a partial antagonist support a role for the Ah receptor in mediating TCDD-induced antiestrogenicity. A construct of an ERE linked to the tk-CAT plasmid was transfected into MCF-7 cells and treated with E2,TCDD, or E2 plus TCDD. E2 induced CAT activity, TCDD did not induce this activity and in the cotreatment group (E2 plus TCDD) TCDD did not significantly suppress the E2 inducibility.These data provide preliminary evidence for the hypothesis that the antiestrogenicity of TCDD is mediated through the Ah receptor-dependent induction of trans-acting gene products.

INTRODUCTION

TCDD and related halogenated aryl hydrocarbons (HAHs) are industrial chemicals or by-products which induce phase I and II metabolizing enzymes such as cytochrome P4501A1 (CYP1A1). The molecular mechanism for TCDD responses is mediated through the aryl hydrocarbon (Ah) receptor which translocates to the nucleus[1,2]. Studies suggest that the TCDD-nuclear Ah receptor complex acts as a transcriptional enhancer which interacts with specific genomic sequences(DRE) and results in increased CYP1A1 gene expression[3-5].

The molecular response mechanism of action for the induction of the CYP1A1 gene provides a working model for describing the mechanisms associated with other TCDD-induced responses, such as antiestrogenicity.
The aryl hydrocarbon (Ah) receptor has been characterized in several human breast cancer cell lines. Gierthy and coworkers first reported that TCDD inhibited E2-induced growth, secretion of tissue plasminogen activator activity and postconfluent focus production in MCF-7 cells[6-7].TCDD exhibits antiestrogenic properties in estrogen-sensitive tissues both *in vitro* and *in vivo* [8-12]. TCDD does not competitively bind to the steroid hormone receptors nor do the steroid hormones bind to the Ah receptor. In these studies, we have attempted to show that the action of TCDD as an antiestrogen occurs at the 5' flanking region of the *Xenopus* vitellogenin A2 gene and that this action is mediated through the Ah receptor.

MATERIALS AND METHODS

Cell culture:Cells are maintained at 37°C in Dulbecco's modified Eagle's media supplemented with 10% fetal calf serum, sodium bicarbonate (3.7 g/l), and penicillin/streptomycin (10000 units/ liter of penicillin and 10 mg/liter of streptomycin). The media used for transfection is phenol red free and the serum is dextran-charcoal treated to remove estrogenic components .
Plasmid:Plasmid DNA is isolated by alkaline lysis and purified by two cesium chloride-ethidium bromide gradient centrifugations[13]. PA2(-821/+14)CAT and (pA2(-821/-87)tk-CAT were gifts from Dr. G.U. Ryffel[14]. ERE-tk-CAT was a gift from Dr. Ming Tsai[15].
Transfection:Cells are transfected by calcium phosphate co-precipitation. Cells are plated (3×10^6 cells in a 10 cm tissue culture plate) and allowed to grow overnight until 30-50% confluency is reached. The media is replaced with transfection media and then, 1 ml of a 2M CaCl, 2x Hepes Buffered Saline (HBS), and 10 ug plasmid DNA mix is prepared, allowed to precipitate for 30 min, and added dropwise to the media. The treated cells are incubated for 4 hr, media is aspirated and a 25% glycerol/serum free-media shock is added for 30 sec. After 2 washes with Hanks Complete Media, transfection media containing E-2(1nM), TCDD(1, 10 or 100nM) or E-2 plus TCDD (in DMSO,<0.1%) is added. Control plates are treated with DMSO alone.
CAT reaction:The CAT plasmid transfected cells are harvested and lysed 40 hr after dosing. The cells are scraped from the plate in 1 ml PBS, and pelleted. The pellet is resuspended in 100 µl of 0.25 M Tris-hydrochloride (Tris-HCl) (ph7.5) and taken through 3 cycles of rapid freezeing and thawing. The CAT reaction mix[16] consists of cell lysate containing 100 µg protein and 0.25M Tris-HCl in a 120 µl volume. One µCi of [^{14}C] chloramphenicol and 40 µl of 4 mM acetyl CoA are added, incubated for 1 hr at 37° C, and the reaction stopped by addition of 750 ul ethyl acetate. Six hundred µl of the ethyl acetate, which contains all the forms of chloramphenicol, are removed, dried, and resuspended in 20 µl ethyl acetate. The samples are spotted on a TLC plate, which is then developed using a 95:5 chloroform/methanol solvent mixture. The radioactivity in the nonacetylated and both monoacetylated chloramphenicol spots are quantitated using a Betascope 603 Blot Analyzer. CAT activity is expressed as percent

acetylated chloramphenicol or as a ratio of counts in the acetylated species/total counts (monoacetylated & nonacetylated).

RESULTS AND DISCUSSION

The result illustrated in Figure 1 shows that TCDD inhibited E-2 induced CAT activity in MCF-7 cells transfected with the PA_2 (-821/+14)-CAT (VIT-CAT) construct. Replacement of the vitellogenin promoter with the thymidine kinase promoter of *Herpes* virus {(-821/-87)tk-CAT} does not affect TCDD suppression of the E2 inducibility.

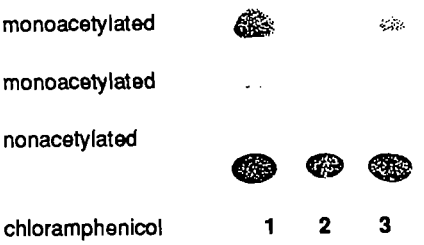

Figure 1: TCDD Inhibition of E2 CAT Induction. Autoradiograph of CAT assay in MCF-7 cells. 1 was dosed with E2 1nM,CAT activity=22%; 2 with TCDD 100nM,CAT=4%; and 3 with E2 plus TCDD, CAT=11%. All are in DMSO, which is <0.2%

Experimental data which support the role of the Ah receptor in mediating antiestrogenicity in MCF-7 cells is as follows. The suppression of estrogen inducibility shows structure activity relationship as demonstrated by the potencies of various Ah receptor agonists (data not shown). TCDD inhibition of estrogen-inducibility is antagonized by α-naphthoflavone which competes for the Ah receptor (Figure2); and in mouse Hepa 1c1c7 cells, TCDD inhibits E2-induced CAT activity in the Ah-responsive wild type but not the Ah receptor translocation-deficient class II mutants (Figure 3)

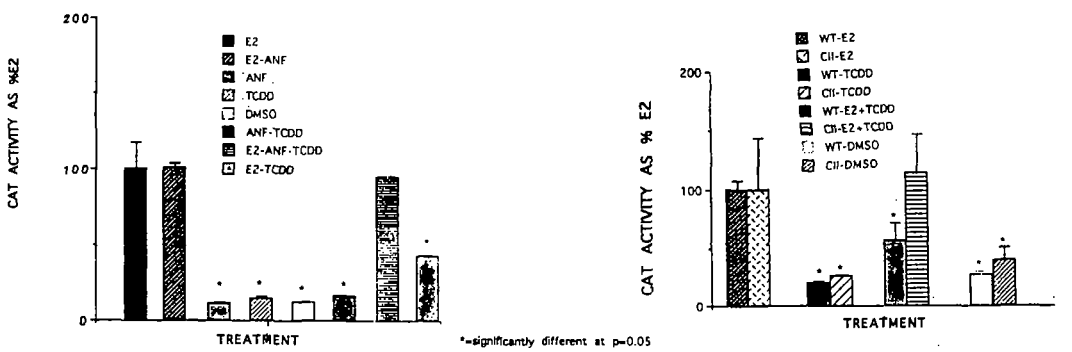

Figure 2: Inhibition of TCDD-Induced Antiestrogenicity by α–Naphthoflavone (ANF) in MFC-7 Cells. ANF competes with TCDD for the Ah receptor.
Figure 3: Inhibition of E2 Induced CAT Activity by TCDD in Mouse Hepa1c1c7 Cells Class II mutants are Ah Receptor translocation deficient.
In both experiments, E2 was dosed at 1nM, TCDD at 100nM, ANF at 1mM in <0.2% DMSO.

Results of transfection of ERE-tk-CAT into MCF-7 cells are shown in Figure 4. As with the pA2(-821/-87)tk-CAT, E2 induces CAT activity, TCDD does not induce this activity, but cotreatment (E2 plus TCDD) does not significantly suppress the E2 induction.

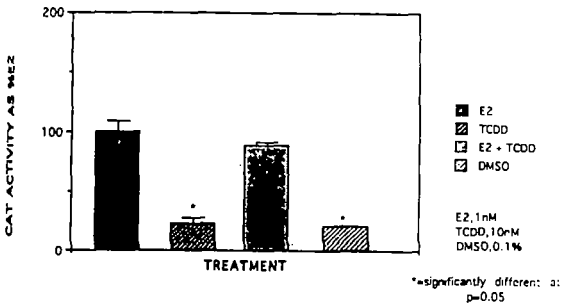

Figure4:TCDD Effect on E2 Inducibility in ERE-tk-CAT Transfected MCF-7 cells

The results support the role of the Ah receptor in mediating TCDD-induced antiestrogenicity in MCF-7 cells and current studies are focused on identifying the *cis*-genomic sequences involved in this response. (Supported by the National Institutes of Health, ES04176).

REFERENCE

1. Poland, A., Glover, E. and Kende, A.S. J. Biol. Chem. 251:4936-4946, 1976.
2. Safe, S. ISI Atlas of Science. Pharmacol. 2:78-83, 1988.
3. Whitlock, J.P. Pharmacol. Rev. 39:147-161, 1987.
4. Whitlock, J.P. Annu. Rev. Pharmacol. Toxicol. 26:333-369, 1986.
5. Safe, S., Gasiewicz, T.A. and Whitlock, Jr., J.P. Environmental Toxin Series, Vol. 3. Springer Verlag, Heidelberg, Germany, pp. 61-91, 1990.
6. Gierthy, J.F., Lincoln, D.W., Gillespie, M.B., Seeger, J.I., Martinez, H. L., Dickerman, H.W. and Kumar, S.A. Cancer Res. 47:6198-6203, 1987.
7. Gierthy, J.F. and Lincoln, D.W. Breast Cancer Res. Treat. 12:227-233, 1988.
8. Kociba, R.J., Keyes, D.G., Beger, J.E., Carreon, R.M., Wade, C.E., Dittenber, D.A., Kalnins, R.P., Frauson, L.E., Park, C.L., Barnard, S.D., Hummel, R.A. and Humiston, C.G. Toxicol. Appl. Pharmacol. 46:279- 303, 1978.
9. Romkes, M., Piskorska-Pliszczynska, J. and Safe, S. Toxicol. Appl. Pharmacol. 87:306-314, 1987.
10. Romkes, M. and Safe, S. Toxicol. Appl. Pharmacol. 92:368-380, 1988.
11. Astroff, B., Rowlands, C., Dickerson, R. and Safe, S. Mol. Cell. Endocrinol.72:247-252,1990.
12.Biegel, L. and Safe,S. J. Steroid Biochem. and Molec. Biol. 37:725-732, 1990.
13. Sambrook,J., Fritsch, E.F. and Maniatis, T. Molecular Cloning, A Laboratory Manual. Cold Spring Harbor Press, Cold Spring HArbor, NY, 1989.
14.Klein-Hitpass,L.,Schorpp,M.,Wagner,U. and Ryffel,G.U.Cell.46:1053-61,1986.
15. Klein-Hitpass,L.,Tsai,S.Y., Greene,G.L.,Clark,J.H., Tsai, M.J., and O'Malley,B.W. Mol. Cell Biol. 9(1):43-49, 1989.
16. Gorman, C.M., Moffat, L.F. and Howard, B.H. Mol. Cell. Biol. 2(9):1044-1051, 1982.