CROSS-TALK BETWEEN 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN AND TESTOSTERONE SIGNAL TRANSDUCTION PATHWAYS IN LNCaP PROSTATE CANCER CELLS

Hideko Sone[1,3], Nihar Ranjan Jana[1], Shubhashish Sarkar[1], Mayumi Ishizuka[2,3], Chiharu Tohyama[2,3] and Junzo Yonemoto[1,3]

[1]Regional Environment Division; and [2]Environmental Health Sciences Division, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-0053, Japan.
[3]CREST, JST(Japan Science and Technology), Kawaguchi, Saitama, 332-0012, Japan

Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds modulate various endocrine functions by enhancing ligand metabolism, altering hormone synthesis, down regulating receptor levels and interfering with gene transcription. Induction of cytochrome P4501A1 (CYP1A1) gene expression by TCDD is considered to be an early and sensitive biochemical response and therefore it has been employed to assess exposure and sensitivity to this compound (1, 2). However, the means by which it modulates the abundance of a number of mRNA's, such as transforming growth factor-α and β, interleukin-1β and plaminogen activator inhibitor -2 are not yet clear (3, 4).

There are a plenty of reports that in the rat and mouse, *in utero* and lactational exposure of TCDD decreases ventral prostate, seminal vescicle and epididymal weights and also the daily sperm production (5-8). Exposure to TCDD, during adulthood, in male rats, also produces similar responses (8-11). Therefore, the effects of adult, *in utero* and lactational TCDD exposure might involve a direct action on the prostate gland. In the present study, we investigated the effects of TCDD on testosterone signal transduction pathways and vice versa in the androgen receptor (AR) positive LNCaP prostate cancer cell line.

Material and Methods

Human prostate cancer cells of the LNCaP-FGC line were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) that had been pre-treated with dextran coated charcoal, 1% glutamine, 100 units penicillin/ml and 100 μg streptomycin/ml under standard conditions, in a 37°C incubator with a humidified mixture of 5% CO_2 and 95% air. For the TCDD dose and kinetic study, cells were treated at about 80-90% confluency on 60-mm^2 dishes with different concentrations of TCDD in 0.1% DMSO (v/v) for different time periods. The controls received 0.1% DMSO alone. For ethoxyresorufin O-deethylase (EROD) assays, cells were homogenized in ice-cold 10 mM Tris-25 mM sucrose buffer and, EROD activities of the aliquot were measured spectoflurometrically as described earlier. In transfections and reporter assays, the cells were transfected with 2 μg of MMTV-LUC (an androgen-responsive reporter plasmid) and 0.02 μg pRL-SV40 plasmid (control plasmid) per dish using lipofactAMINE plus reagent. Luciferase activity was analyzed for 15 sec in 20 μl cell extracts with dual luciferase reporter assay system (Promega, USA) on a TD 20/20 luminometer (Turners Design) and data were presented relative to the ratio of Firefly to Renilla luciferase. Total RNA was prepared from cells using Isogen (Nippon Gene, Japan) according to the manufacturer's instruction and RT-PCR was carried out with an RT-PCR kit (TaKaRa Biomedicals, Japan).

Results and Discussion

TCDD induced CYP1A1 mRNA and related enzyme activity in these cells, in dose and time-dependent manners. Both normal and testosterone-stimulated cell growth was inhibited by TCDD. The expression levels of the aryl hydrocarbon receptor (AhR), the aryl hydrocarbon receptor nuclear translocator (ARNT) and AR were not affected by exposure to TCDD at a dose of 10 nM for a 24 hr time period. Treatment with testosterone alone had no effects on CYP1A1 expression in the LNCaP cells. However, co-administration of testosterone with 100 nM TCDD dose-dependently inhibited TCDD-induced CYP1A1 mRNA accumulation and EROD activity (Fig.1). Testosterone at an equimolar concentration (both 100 nM) inhibited TCDD-induced CYP1A1 mRNA accumulation (as estimated from densitometric scanning) and EROD activity by 55 and 67% respectively.

Next, we studied effects of TCDD on normal and testosterone-induced transcriptional activity in a transiently transfected androgen-responsive reporter assay system (Data not shown). Testosterone at a dose of 10 nM stimulated the androgen-responsive reporter gene activity about 37-fold, and this was reduced to 29- and 10- fold respectively by 10 and 100 nM of TCDD. TCDD at a dose of 1nM along with 10 nM testosterone had no inhibitory effects; rather it possibly had a slight stimulatory effect on androgen-responsive reporter gene activity. To further confirm the down-regulation of testosterone response by TCDD, we also studied the testosterone-regulated expression of endogenous PSA in the cells. PSA mRNA and protein levels (cellular content and release) were both strongly elevated upon the addition of testosterone and this elevation was inhibited by 10 and 100 nM TCDD. TCDD alone at a dose of 100 nM also exerted a suppressing effect on constitutive PSA mRNA and protein levels.

The prostate gland is a major male accessory sex organ whose growth, development and normal function depend on androgens. The present study demonstrated the inhibitory effects of TCDD on prostate cancer cell growth. It is therefore possible that in prostate cancer cells, TCDD inhibits cell growth by interfering with growth factor signaling pathways or by altering cell-cycle regulatory proteins downstream of the androgen receptor action. Indeed TCDD was here found to interfere with testosterone-induced transcriptional activity and also testosterone-regulated PSA expression. While it is possible that TCDD might interact directly with AR to produce antiandrogenic influence, it might equally be able to interact with androgen-responsive *cis*-acting elements called androgen response elements (ARE) through liganded AhR-ARNT complexes.

Induction of xenobiotic metabolizing enzymes such as CYP1A1 is considered to be the most sensitive indicator of TCDD exposure and an early event in toxic or carcinogenic responses. In normal prostate cancer cells, CYP1A1 mRNA and related enzyme activity were not at all detectable, but they were induced dramatically upon exposure to TCDD, in line with an *in vivo* report (12) demonstrating prostate involvement in drug metabolism including induction of CYP1A1. While AhR and its dimerization partner ARNT, which are mainly responsible for TCDD-mediated CYP1A1 induction, were here found to be expressed in LNCaP cells and their expression was not altered by TCDD exposure. This contrasts with *in vivo* and *in vitro* demonstration of down- regulation of AhR by TCDD (12, 13). The dramatic dose-dependent inhibition of TCDD-induced CYP1A1 mRNA accumulation and related enzyme activity by testosterone found here is consistent with the findings of others suggesting similar inhibitory effects of estradiol or progesterone. Since, neither testosterone nor dihydrotestosterone (DHT) is able to displace TCDD from AhR (14), it can be expected that the mechanism of this interference is indirect, and possibly 1) by interaction of liganded AR with XREs or 2) by interaction of

liganded AR with liganded AhR.ARNT complexes or other transcription factors which are responsible for TCDD-mediated gene transcription.

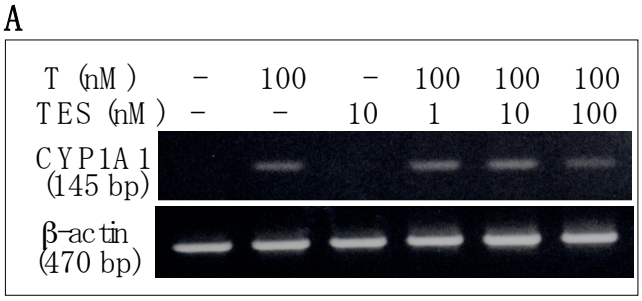
In conclusion, our present observations clearly demonstrate a potent antiandrogenic effect of TCDD and bilateral transcriptional interference between testosterone and TCDD-induced signal transduction pathways in LNCaP prostate cancer cells.

Acknowledgment

We thank Dr. K. Umesono, Institute for Virus Research, Kyoto University, for kindly providing the MMTV-LUC reporter plasmid. This work was supported in part by the Science and Technology Agency fellowship to N.R.J. and S.S.

References

1. Nebert, D. W. and Jones, J. E. (1989) *Int. J. Biochem*., 21, 243-252.
2. Safe, S. H. (1994) *Crit. Rev. Toxicol*., 24, 87-149.
3. Sutter, T. R., Guzman, K., Dold, K. M. and Greenlee, W. F. (1991) *Science*, 254, 415-418.
4. Choi, E. J., Toscano, D. G., Ryan, J. A., Riedel, N. and Toscano, W. A. Jr. (1991) *J. Biol. Chem*., 258, 10731-10737.
5. Mably, T. A., Moore, R. W. and Peterson, R. E. (1992) *Toxicol. Appl. Pharmacol*., 114, 97-107.
6. Theobald, H. M. and Peterson, R. E. (1997) *Toxicol. Appl. Pharmacol*., 145, 124-135.
7. Moore, R. W., Potter, C. L., Theobald, H. M., Robinson, J. A. and Peterson, R. E. (1985) *Toxicol. Appl. Pharmacol*., 79, 99-111.
8. Gray, L. E., Kelce, W. R., Monosson, E., Ostby, J. S. and Birnbaum, L. S. (1995) *Toxicol. Appl. Pharmacol*., 131, 108-118.
9. Prins, G. S., Birch, L. and Greene, G. L. (1991) *Endocrinology*, 129, 3187-3199.
10. Johnson, L., Dickerson, R., Safe, S. H., Nyberg, C. L., Lewis, R. P and Welsh, T. H. (1992) *Toxicology*, 76, 103-118.
11. Roman, B. L., Sommer, R. J., Shinomiya, K. and Peterson, R. E. (1995) *Toxicol. Appl. Pharmacol*., 134, 241-250.
12. Roman, B. L., Pollenz, R. S. and Peterson, R. E. (1998) *Toxicol. Appl. Pharmacol*., 150, 228-239.
13. FitzGerald, C. T., Fernandez-Salguero, P., Gonzalez, F. J., Nebert, D. and Puga, A. (1996) *Arch. Biochem. Biophys*., 333, 170-178.
14. Poland, A., Glover, E. and Kende, A. S. (1976) *J.Biol. Chem*., 251, 4936-4946.



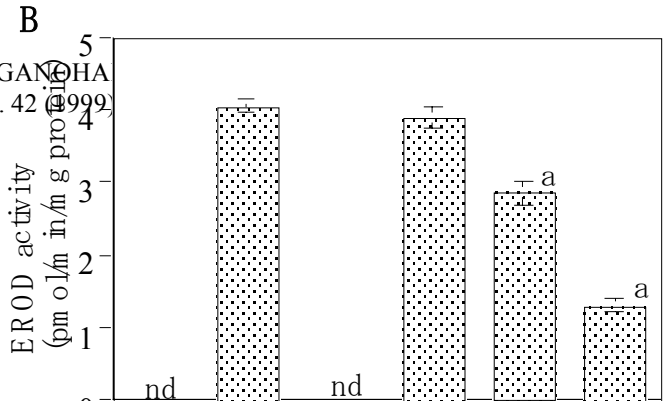


Fig.1. Inhibition of TCDD-stimulated CYP1A1 mRNA expression and EROD activity by testosterone. LNCaP cells were incubated with TCDD (T) or testosterone (TES) alone or in different combinations for 24 hrs and then processed for RT-PCR of CYP1A1 (A) and EROD assays (B) according to the procedures described in the Materials and Methods. Result are means ± SD of three independent experiments, each performed in triplicate. a; $p<0.001$ as compared to the TCDD treated group.