Effects of 2,3,7,8 Tetrachlorodibenzo-p-dioxin on Insulin-like Growth Factor Binding Protein 4 in MCF-7 and T47D Human Breast Cancer Cells

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1. Introduction

Human breast cancer cell are mitogenically responsive to insulin-like growth factors (IGFs) [1)] which exert their actions through autocrine and paracrine pathways [2)]. This mitogenic activity is modulated by IGF binding proteins (IGFBPs), which either enhance or inhibit the action of IGF by binding to IGF [3)]. Six IGFBPs have been characterized and cloned. Human breast cancer cells express four of the six IGFBPs in a pattern that correlates with the estrogen-responsiveness of the cells. The altered production of the IGFBPs by breast tumors may influence malignant growth by interfering with IGF-mediated responses [2)]. Pratt and Pollak have characterized the effects of estrogen and antiestrogens on IGFBP expression in the MCF-7 breast cancer cell line. The levels of secreted IGFBPs demonstrated a complex pattern in response to estrogen. Specifically, E2 was shown to induce secretion of IGFBP 4, while the antiestrogens, tamoxifen and ICI 182,780, reduce constitutive and E2-induced levels of IGFBP 4 secretion [1)].

TCDD elicits a number of biochemical and toxic effects including inhibition of several estrogen-induced responses in both *in vivo* and *in vitro* models. For example in MCF-7 cells, TCDD inhibits E2-induced cell proliferation and secretion of the 34-, 52- and 160-kDa proteins [4,5,6,7)]. Since IGFBP 4 is downregulated by antiestrogens, the effects of TCDD on the 17β-estradiol (E2)-induced secretion of this protein in MCF-7 and T47D cells was investigated. The effects of TCDD on E2-induced IGFBP 4 gene expression were also investigated.

2. Methods

Treatment of Cells. The human breast cancer MCF-7 and T47D cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD). Cells were maintained in DME/F12 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.

Western Ligand Blot Analysis. Cells were treated with DMSO, TCDD, E2 or combinations before conditioned media was collected. Western ligand blot analysis was carried out as previously described by Hossenlopp and coworkers [8)] using [^{125}I]IGF-I (8.0 to 12.0 x 10^5 cpm) (Amersham). The data was quantitated using a Molecular Dynamics Computing

Densitometer.

Northern Analysis. The plasmid pHBP4-503 containing the IGFBP 4 cDNA was obtained from Dr. Shunichi Shimasaki (The Whittier Institute for Diabetes and Endocrinology, LaJolla, CA). RNA was extracted from the cells treated with DMSO (control), TCDD, E2 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 prepared and probed as described earlier by Krishnan and coworkers [9].

3. Results and Discussion

The data presented in Figure 1 illustrate the effects of E2 and TCDD on secretion of IGFBP 4 in MCF-7 cells. E2 (1 nM) caused an approximate 2-fold increase in secretion of IGFBP 4. TCDD (10 nM) also significantly downregulated constitutive and E2-induced IGFBP 4 secretion. As shown in Figure 2, E2 (10 nM) caused a time-dependent increase in IGFBP 4 mRNA levels in MCF-7 cells with a maximal 2-fold increase observed after 24 hr. TCDD (10 nM) caused a significant decrease in E2-induced IGFBP 4 mRNA levels within 2 hr after treatment and the inhibitory response persisted for 24 hr. This data is consistent with other studies showing that TCDD inhibits diverse E2-induced responsive in human breast cancer cells [8].

The results in Figure 3 show that E2 (1nM) caused an approximate 2-fold increase in secretion of IGFBP 4 in T47D cells. TCDD (10 nM) also significantly downregulated E2-induced levels of IGFBP 4. Preliminary results show that IGFBP 4 mRNA levels are also decreased in T47D cells within 2 hr after treatment with TCDD. These data were comparable to those observed in MCF-7 cells and suggest similar IGFBP 4 regulation in both cell lines.

Previous studies in this laboratory have shown that TCDD inhibits E2-induced cathepsin-D gene expression by direct interaction of the nuclear aryl hydrocarbon receptor (AhR) complex with genomic dioxin responsive elements (DREs) in the 5'-promoter region of the gene [9]. Gierthy and coworkers proposed that the antiestrogenic effects of TCDD are related to induced P450 which increases the metabolism of E2 leading to a cellular depletion of the hormone [10]. The early antiestrogenic effects of TCDD (within 2 hr) on IGFBP 4 mRNA levels indicate that E2 metabolism does not play a role in the downregulation of IGFBP 4 mRNA since the P450 induction response is not observed until 3 to 6 hr after TCDD treatment. The molecular mechanism associated with aryl hydrocarbon receptor-mediated inhibition of E2-induced IGFBP 4 is currently being investigated.

4. Acknowledgements

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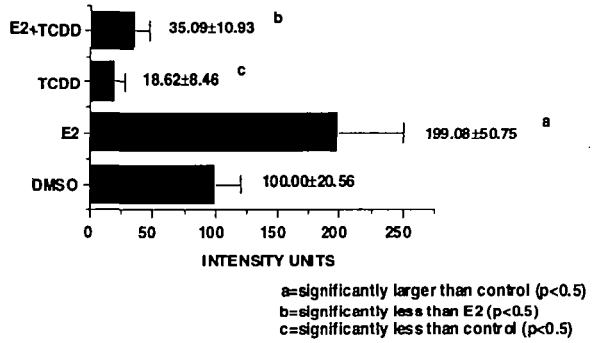

Figure 1. Downregulation of IGFBP4 Secretion in MCF-7 Cells

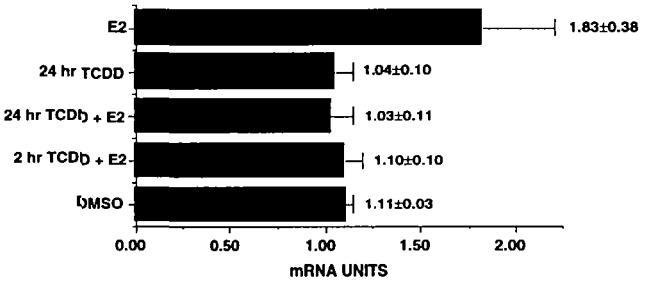

Figure 2. Downregulation of IGFBP 4 mRNA by TCDD in MCF-7 Cells

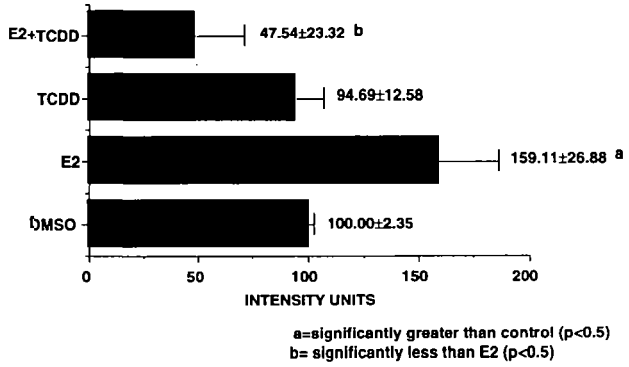

Figure 3. Downregulation of Secretion of IGFBP 4 by TCDD in T47D Cells

5. References

1. Pratt, S. and Pollak, M. (1993) Estrogen and antiestrogen modulation of MCF-7 human breast cancer cell proliferation is associated with specific alterations in accumulation of insulin-like growth factor-binding proteins in conditioned media. *Cancer Res.* 53:5193.
2. Figueroa, J. and Yee, D. (1992) The insulin-like growth factor binding proteins (IGFBPs) in human breast cancer. *Breast Cancer Res. Treat.* 22:81.
3. Pekonen, F., Nyman, T., Ilvesmäki, V. and Partanen, S. (1992) Insulin-like growth factor binding proteins in human breast cancer tissue. *Cancer Res.* 52:5204.
4. Fernandez, P. and Safe, S. (1992) Growth inhibitory and antimitogenic activity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in T47D human breast cancer cells. *Toxicol. Lett.* 61:185.
5. Merchant, M., Morrison, V., Santostefano, M. and Safe, S. (1992) Mechanism of action of aryl hydrocarbon receptor antagonists: inhibition of 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin-induced CYP1A1 gene expression. *Arch. Biochem. Biophys.* 298:389.
6. Landers, J. and Bunce, N. (1991) The *Ah* receptor and the mechanism of dioxin toxicity. *Biochem. J.* 276:273.
7. Biegel, L. and Safe, S. (1990) Effects of 2,3,7,8 tetrachlorodibenzo-*p* -dioxin (TCDD) on cell growth and the secretion of the estrogen-induced 34-, 52-, and 160-kDa proteins in human breast cancer cells. *J. Steroid Biochem. Mol. Biol.* 37:725.
8. Hossenlopp, P., Seurin, D., Segovia-Quinson, B., Hardouin S. and Binous, M. (1986) Analysis of serum insulin-like growth factor binding proteins using western blotting: use of the method for titration of the binding proteins and competitive binding studies. *Anal. Biochem.* 154:138.
9. Krishnan, V., Wang, X. and Safe, S. (1994) Estrogen receptor-Sp1 complexes mediate estrogen-induced cathepsin-D gene expression in MCF-7 human breast cancer cells. *J. Biol. Chem.* 269:15912.
10. Gierthy, J., Lincoln, D., Kampcik, S., Dickerman, H., Bradlow, H., Niwa, T., Swaneck, G. (1988) Enhancement of α- and 16α-estradiol hydroxylation in MCF-7 human breast cancer cells by 2,3,7,8 tetrachlorodibenzo-*p*-dioxin. *Biochem. Biophys. Res. Commun.* 157:515.