Implication of Growth Factor and Growth Factor Receptor Modulation in Dioxin Toxicity

Burra V. Madhukar
Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN 46202, U.S.A.

1. Introduction

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin) and isosteric environmental pollutants evoke pleiotropic toxic responses in many species of experimental animals. The variety of toxicities elicited in animals treated with TCDD include enzyme induction, hepatotoxicity, reproductive toxicity, embryotoxicity, teratogenicity, immunosuppression, tumor promotion and carcinogenicity.[1] Indeed, TCDD is one of the most potent teratogens known that causes cleft palate and hydronephrosis.[2] The extremely low concentrations at which dioxin causes toxicity suggested that this and structurally similar xenobiotics might be acting through binding to a receptor.[3] Subsequently Poland and associates have identified a hepatic cytosolic receptor protein, the Ah receptor, to which TCDD binds.[4] They also demonstrated that the teratogenic effects of TCDD to inbred strains of mice is related to the affinity of Ah receptor expressed to bind with TCDD.[5] In the less sensitive (nonresponsive) strains such as the DBA/2J cleft palate could only be induced at much higher concentrations of TCDD than were required for the senstive (responsive) strains of mice such as the C57BL/6J . Now we know much more about the receptor and its mechanism of activation. Accordingly, the Ah receptor is a member of the steroid superfamily of receptors but is distinct from the other steroids hormone receptors. There is yet no known endogenous ligand for Ah receptor. It has been suggested that most, if not all, of the toxic responses mediated by TCDD and isosteric xenobiotics are a consequence of their binding to the Ah receptor.[6] The ligand-bound Ah receptor undergoes activation (transformation) and translocates to the nucleus where in association with a nuclear protein termed Ah receptor nuclear translocator protein (ARNT) is able to bind with specific sequences (enhancer regions) called dioxin response elements (DREs) or xenobiotic response elements within the promoter elements of responsive genes.[7,8] Thus the Ah receptor functions as a ligand-induced transcription factor to regulate gene transcription in a manner similar to steroid hormones. Among the genes regulated by dioxin and similar agents are cytochrome P-450 cyp-1a1, transforming growth factor-α (TGF-α), plasminogen activator inhibitor-2, and interleukin-1b (reviewed in reference 9). The toxic responses evoked by dioxins are, therefore, likely to involve transcriptional modulation of critical genes involved in cell proliferation and differentiation. While the Ah receptor presence and its binding with dioxin (or other logands) are essential for the elicitation of any toxic responses, it is evident from many reports that the subsequent biochemical/molecular changes that occur in responsive tissue may also play a major role in the toxicity of dioxin. Since many proteins undergo reversible phosphorylation for functional activation (via phosphorylation) and inactivation (via dephopshorylation) we proposed that in dioxin responsive tissue such as the liver and the skin *in vivo* and in *in vitro* systems such as the mouse teratocarcinoma cell line, XB, dioxin modulates the phosphorylation state of membrane and other cellular proteins which may paly a role in the tissue specific toxicities of this agent.[10] These studies were undertaken to examine dioxin effects in hepatocyte membrane protein changes after *in vivo* treatment of rats with a single dose of dioxin, or after *in vitro* treatment of cultured cells with dioxin.

2. Results

One of the major alterations induced by *in vivo* exposure of laboratory animals such as rats and mice was a marked decrease in the number of EGF receptors (EGFR) from the hepatic plasma membrance first demonstrated by us.[11] In the mouse strains the down regulation of EGF binding directly correlated with the affinity of the Ah receptor of the strains.[12] Our initial focus was on the hepatic tissue since dioxin is a known hepatotoxin and liver tumor promoter. Examination of the plasma membrane proteins and changes in the phosphorylation of membrane associated proteins revealed that a number of them showed enhanced phosphorylation. Since we also observed that dioxin decreased the binding of EGF to the plasma membrane we examined whether this was due to modulation of EGFR phosphorylation. The EGFR has intrinsic tyrosine kinase activity that is induced upon EGF binding and internalization of the ligand bound receptor. Therefore, we postulated that TCDD induced down regulation of EGF receptor could be due to the induction of tyrosine kinase activity that led to the internalization of the receptor with a consequent loss of EGF binding to the cell surface membrane. Since TCDD itself does not compete with EGF it is not a direct ligand for the EGFR. To further test whether TCDD modulation of EGFR results in EGF like effects *in vivo*, we administered TCDD to neonatal Sprague Dawley rats to examine whether such treatment causes biological effects of EGF such as early eye lid opening and precocious tooth eruption. The results clearly demonstrated that *in vivo* TCDD mimics the biological effects of EGF.[11] To substantiate further that the effect of TCDD on EGFR are direct and are not mediated through other mechanisms *in vivo*, we studied TCDD down regulation of EGF binding in the XB cells derived from mouse teratocarcinoma.[13] Treatment of XB cultures with TCDD caused an increase in the tyrosine phosphorylation of a number of membrane proteins including that of the EGFR and led to keratinization of the cultures in the presence of irradiated fibroblasts. These observations have led us to consider that TCDD treatment *in vivo* or *in vitro* could induce the activity of another kinase or the production of a peptide growth factors that could phosphorylate EGFR.

3. Discussion

Two questions that must now be considered are: 1) what are the mechanism(s) through which dioxin causes protein phosphorylation and 2) how protein phosphorylation induced by dioxin is involved in the toxicological and cellular effects. Obviously the answer to the first question is that somehow dioxin treatment increases the activity of kinases, either serine and threonine or tyrosine, to induce the phosphorylation of substrate proteins. Alternatively, dioxin treatment could lead to enhanced expression of growth factors which, in turn, induce the kinase activities of their respective receptors through increased binding. This possibilitiy is supported by the observations that dioxin treatment induces the expression of TGF-α in the keratinocytes and palatal epithelial cells in culture.[14] This increase could cause EGF receptor internalization with a concomittant increase in its kinase activity. Since EGF and TGF-α are mitogenic growth factors and bind with the same receptor, the sustained increase in EGFR kinase activity could provide the stimulus for increased cell proliferation as a result of phosphorylation of key proteins in the mitogenic signalling pathway. The inapporpriate signalling of this pathway induced by treatment with dioxin appears to be involved in the induction of cleft palate by interfering with the programed differentiation of palatal epithelium.[15] There is some evidence that the calcium and phospholipid dependent serine, threonine protein kinase C (PKC) might be involved in the phosphorylation of the Ah receptor.[16] Several other investigators were unable to show PKC activation by dioxin and it thus appears that the promoting effects of dioxin differ from those of the phorbol ester, TPA. Indeed dioxin has been shown to be 100-fold more potent than the phorbol ester, TPA as a promoter of skin tumors in the hr/hr homozygous hairless mice.[17] However, we cannot rule out the possibility that dioxin exposure may cause the activation of PKC isozymes that are independent of calcium and were not detectable using the classical histone phosphorylation assay. It may thus be worthwhile to use other techniques to examine the effect of TCDD on specific isozymes of PKC. Further evidence that tyrosine kinase activation may be induced by dioxin also comes from the observations by Matsumura and associates which demonstrated activation of nonreceptor tyrosine kinase

activity of c-src protooncogene and phosphoproteins in adipocytes after dioxin treatment.[18,19]

A question that has not yet been answered is whether all of the toxic effects of dioxin are mediated only through Ah receptor or whether there are any biological and toxicological effects that are independent of Ah receptor. From all the research that had been done so far it appears that binding of TCDD with the Ah receptor is an initial first step that must occur for the elicititation of any biological effects. Once this happens, the spectrum of biological and toxicological effects that occur in a tissue specific manner depends on the battery of genes that are activated. At this point there is a bifurcation of the effects of TCDD as depicted in Figure 1. If one of the battery of genes that are affected by TCDD is a kinase and is part of the signal transduction cascade involved in cell growth and differentiation, then, the biological effects of TCDD exposure are more pronounced and varied. The activation of a kinase or kinases by TCDD can not only phosphorylate plasma membraneassociated proteins including the EGFR but may also cause the activation of other transcription factors and nuclear prtoeins that are involved in cell growth and differentiation. It should be interesting, in future investigations, to examine whether theAh receptor expression can be abrogated with antisense Ah receptor cDNA constructs transfected into

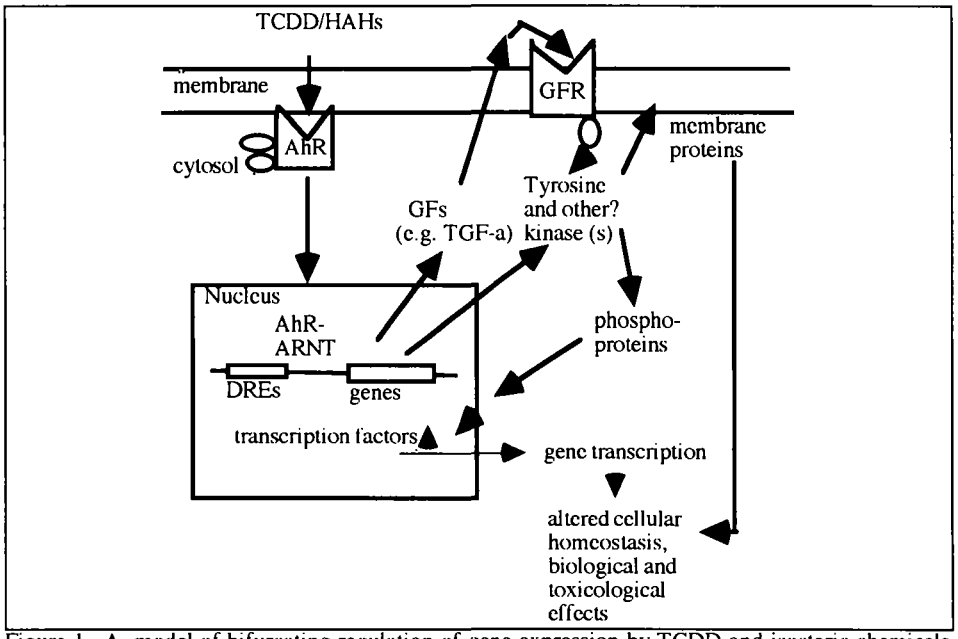

Figure 1. A model of bifurcating regulation of gene expression by TCDD and isosteric chemicals. TCDD binds with the cytosolic Ah receptor, AhR and this binding transforms the receptor which then translocates to the nucleus in association with the Ah receptor nuclear translocator protein, ARNT. This complex recognizes specific enhancer sequences, the DREs of responsive genes to induce their transcription. A tyrosine protein kinase or a kinase kinase may thus be expressed to phosphorylate other kinases and/or substrate proteins such as transcription factors (e.g. AP-1). This activation, in turn, induces the transcription of other genes regulated by them. In some cases the genes for growth factors (GF) may also be activated by TCDD causing ligand induced receptor (GFR) activation and phosphorylation of cellular proteins.

TCDD responsive cell culture systems and whether such models still elicit biological effects in response to TCDD.

4. REFERENCES

1) Vanden Heuvel J.P., and G. Lucier (1993) : Environmental toxicology of polychlorinated dibenzo-p-doxins and polychlorinated dibenzofurans, Environ. Hlth. Persp. 100, 189-200
2) Poland A., and J.C. Knutson (1982) : 2,3,7,8-tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbon:Examination of the mechanism of toxicity, Ann. Rev. Pharmcol. Toxicol. 22, 517-554
3) Poland A., and E. Glover (1973) : Comparison of 2,3,7,8-tetrachlorodibenzo-p-dioxin, a potent inducer of aryl hydrocarbon hydroxylase, with 3-methylcholanthrene, Mol. Pharmacol. 10, 349-359
4) Poland A., E. Glover, and A.S. Kende (1976) : Stereospecific, high affinity binding of TCDD by hepatic cytosol: Evidence that the binding species is the receptor for induction of arylhydrocarbon hydroxylase, J. Biol. Chem. 259, 4936-4946
5) Poland A., and E. Glover (1980) : 2,3,7,8-tetrachlorodibenzo-p-dioxin:Seggregation of toxicity with the the Ah locus, Mol. Pharmacol. 17, 86-94
6) Okey A.B., D.S. Riddick and P.A. Harper (1994) : The Ah receptor: Mediator of the toxicity of 2,3,7,8-tetrachlorodibenzo-p-pdioxin (TCDD) and related compounds, Tox. Lett. 70, 1-22
7) Reyes H., S. Reisz-Porszasz, and O. Hnakinson (1992) : Identification of the Ah receptor nuclear translcoator protein (Arnt) as a component of the DNA binding form of the Ah receptor, Scinece 256, 1193-1194
8) Whitlock J.P. Jr. (1993) : Mechanistic aspects of dioxin action, Chem. Res. Toxicol. 6, 754-763
9) Greenlee W.F., T.R. Sutter, and C. Marcus (1994) : Molecular basis of dioxin actions on rodent and human target tussies, in:Receptor-mediated biological processes:Implications for evaluating carcinogenesis, H.L. Spitzer, W.F. Greenlee and R.M. McClain (eds.), pp.47-57, Wiley-Liss, Inc. New York
10) Matsumura F. (1994) : How important is the protein phosphorylation pathway in the toxic expression of dioxin-type chemicals? Biochem. Pharmacol. 48, 215-224
11) Madhukar B.V., D.W. Brewster, and F. Matsumura (1984) : Effects of in vivo administered 2,3,7,8-tetrachlorodibenzo-p-dioxin on receptor binding of epidermal growth factor in the hepatic plasma membrane of rat, guinea pig and hamster, Proc. Natl. Acad. Sci. USA, 81, 7407-7411
12) Madhukar B.V., K. Ebner, F. Matsumura, D.W. Bombick, D.W. Brewster, and T. Kawamoto (1988) : 2,3,7,8-tetrachlorodibenzo-p-dioxin causes an increase in protein kinases associated with epidermal growth factor receptor in the hepatic plasma membrane, J. Biochem. Toxicol. 3, 261-277
13) Kawamoto T., F. Matsumura, B.V. Madhukar, and D.W. Bombick (1989) : Effects of TCDD on the EGF receptor of XB mouse keratinizing epithelial cells, J. Biochem Tox. 4, 173-182
14) Gaido K.W., S.C. Maness, and W.F. Greenlee (1992) : 2,3,7,8-tetrachlorodibenzo-p-dioxin dependent regulation of transforming growth factors-a and -b_2 expression in a human keratinocyte cell line involves both transcriptional and post-transcriptional control, J. Biol. Chem. 267, 24591-24598
15) Abbott B.D., and LS. Birnbaum (1990) : TCDD-induced altered expression of growth factors may have a role in producing cleft palate and enhancing the incidence of clefts after coadministration of retinoic acid and TCDD, Toxicol. Appl. Pharmacol. 106, 418-432
16) Carrier F., R.A. Owens, D.W. Nebert, and A. Puga (1992) : Dioxin-dependent activation of murine Cyp1a-a gene transcription requires protein kinase C-dependent phosphorylation, Mol. Cell. Biol. 12, 1856-1863
17) Poland A., D. Palen, and E. Glover (1982) : Tumor promotion by 2,3,7,8-tetrachlorodibenzo-p-dioxin in skin of HRS/J hairless mice, Nature (London) 300, 271-273
18) Bombick D.W., J. Jankun, K. Tullis, and F. Matsumura (1988) : 2,3,7,8-tetrachlorodibenzo-p-dioxin causes increases in expression of c-erb-A and levels of protein-tyrosine kinases in selected tissues of responsive mouse strains, Proc. Natl. Acad. Sci. USA 85, 4128-4132
19) Enan E., and F. Matsumura (1994) : Significance of TCDD-induced changes in protein phosphorylation in the adipocyte of male guinea pigs, J. Biochem. Toxicol. 9, 159-170