Tumor Promotion By 2,3,7,8,-Tetrachlorodibenzo-p-dioxin (TCDD) In The Rat Liver: Dose-Response Relationships For TCDD Mediated Effects

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TCDD and its structural analogs cause a variety of biochemical and toxic effects. In chronic bioassays for carcinogenicity TCDD is also a potent hepatocarcinogen in female rats with an increased tumor incidence at a dose of 10ng/kg/day [1]. The mechanism of action is not known but most, if not all, effects of TCDD require an initial interaction with the Ah-receptor [2]. There is controversy regarding dose-response relationships for many toxic and carcinogenic effects of TCDD and its structural analogs. To improve current risk-assessment for dioxins more information on dose-response relationships for Ah-receptor mediated effects is necessary.

We investigated dose-response relationships for various effects of TCDD in a chronic tumor promotion model in female Sprague-Dawley rats. The animals were treated with an initiating dose of diethylnitrosamine (175 mg/kg), then promoted for 30 weeks with different doses of TCDD through biweekly oral gavage equivalent to a daily dose of 0.1 to 125 ng/kg/day. We analyzed TCDD concentrations in the liver, quantified cell proliferation by incorporation of BRDU in DNA, and measured enzyme altered foci. Induction of CYP1A1 and CYP1A2 was quantified by radioimmunoassay and both isozymes were localized in the same livers by immunohistochemical techniques. In addition alterations in epidermal growth factor receptor (EGFR) were investigated using equilibrium binding studies and immunohistochemistry. Estrogen receptor was localized and quantified in the livers by in situ hybridization methods.

Induction of cytochrome P450 isozymes is one of the most sensitive responses to TCDD exposure and is mediated through the Ah-receptor. CYP1A1 and CYP1A2 induction revealed a linear relationship to administered dose as well as to target tissue dose in the dose range from 3.5 to 125 ng/kg/day. For both isozymes immunohistochemical detection showed the same localization and induction pattern characterized by a non-uniform staining even at the highest dose. This indicates that hepatocytes are heterogenious in their response to TCDD.

TCDD concentration in the liver revealed a linear relationship ($r=0.999$) to the administered dose in the dose range from 3.5 to 125 ng/kg/day. The lipid-adjusted TCDD levels also exhibited a linear relation to the administered dose ($r=0.993$). This indicates that induction of cytochrome P4501A2 which binds

TCDD[3] does not significantly alter the tissue distribution in a chronic exposure model.

Cell proliferation was quantified by incorporation of BRDU in DNA. A statistically significant increase over control values was detected only in the highest dose group (125ng/kg/day). The marker for enzyme altered foci was placental gluthathion-S-transferase (PGST). A statistically significant increase in the percentage of liver occupied by PGST positive foci and mean volume of foci was detected in the highest dose group. There was considerable interindividual variation in the effects of TCDD on cell proliferation and presumably preneoplastic lesions.

Scatchard analysis of EGFR binding capacity revealed a dose-dependent decrease in the number of plasma membrane binding sites for EGF. Immunohistochemical methods showed a decreased overall staining for EGFR in cell mambranes accompanied by increased staining in the cytoplasm. The EGFR and ER pathways are known to stimulate mitogenesis in the liver thus suggesting the involvement of these altered growth control pathways in TCDD mediated hepatocarcinogenesis. Current research is attempting to localize EGFR and ER in preneoplastic cells and compare to normal cells.

The mechanistic relationship between enzyme induction, decrease in plasma membrane EGFR binding capacity and cancer is unclear. Nevertheless, mathematical analysis of our data[4] show no evidence for a threshold of these TCDD-mediated effects within the framework of a tumor promotion model.in the dose range of our study. We are currently investigating various parameters in a dose range between 0.1 to 3.5 ng/kg/day in order to more clearly define the shape of the dose response curve in the low dose region including effects produced by background exposure for humans of 1-3 pg/kg/day.

REFERENCES

1. Kociba R, Keyes D, Beyer J, Carreon R, Wade C, Dittenber D, Kalnins R, Frauson L, Park C, Barnard S, Hummel R, Humiston C. Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. *Toxicol. Appl. Pharmacol.* 1978;46:279-303.

2. Whitlock J. Genetic and molecular aspects of 2,3,7,8-tetrachlorodibenzo-p-dioxin action. *Annu Rev Pharmacol Toxicol.* 1990;30:251-277.

3. Voorman R, Aust S. Specific binding of polyhalogenated aromatic hydrocarbon inducers of cytochrome P-450d to the cytochrome and inhibition of its estradiol 2-hydroxylase activity. *Tox. Appl. Pharmacol.*1978;90:69-78.

4. Portier C, Tritscher A, Kohn M, Sewall C, Clark G, Edler L, Hoel D, Lucier G. Ligand/Receptor binding for 2,3,7,8-TCDD: Implications for risk assessment. *Fundamental and Appl. Toxicol.* 1992; in press.

TABLE 1: Summary of TCDD mediated effects in a chronic liver tumor promotion model. All animals were initiated with a single dose of diethylnitrosamine (175 ng/kg) and treated with various doses of TCDD by biweekly oral gavage.

	DOSE TCDD (ng/kg/day)							
	0	0.1	0.3	1	3.5	10	35	125
liver TCDD[1]	0				0.6	1.7	6.7	19.9
BRDU-LI[2]	5.3				3.3	2.9	6.4	12.7
PGST+ foci[3]	0:57				0.85	1.0	0.93	2.23
CYP1A1[4]	1.5				34	130	244	331
CYP1A2[4]	30				87	128	233	388
EGFR[5]	537				569	482	344	207

1 ppb
2 labelling index; percentage of cells which have undergone replicative DNA synthesis in one week
3 Volume fraction; percentage of liver occupied by PGST positive foci
4 pmol/mg microsomal protein
5 Bmax (fmol/mg membrane protein)