ROLE OF ESTROGENS IN LIVER TUMOR PROMOTION BY TCDD IN RATS

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ABSTRACT: 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potent hepatocarcinogen in rodents. However, liver tumor incidence is increased by TCDD in female rats but not male rats. Our studies have investigated this finding by evaluating histological and biochemical parameters in a two-stage model for hepatocarcinogenesis in female rats using diethylnitrosamine (DEN) as the initiating agent and TCDD as the promoting agent. Increases in preneoplastic foci were detected in intact rats and to a lesser extent in ovariectomized rats. This finding was consistent with the cell proliferation data which demonstrated that TCDD markedly increased the labelling index of hepatocytes only in intact rats. These data suggest that ovarian hormones (probably estrogens) play a significant role in the hepatocarcinogenic actions of TCDD.

INTRODUCTION

Chronic bioassays for the carcinogenicity of TCDD have revealed that TCDD is a potent hepatocarcinogen in female rats but not male rats (1,2). TCDD is considered a non genotoxic carcinogen since it does not form DNA adducts and is negative in short term tests for genetic toxicity (3). It is also generally accepted that TCDD is a tumor promoter in skin (4) and liver (5,6). There is compelling evidence in the scientific literature that the Ah receptor is required for the toxic and biochemical (including cytochrome P-450 induction) effects of TCDD (7).

The studies reported here used a two-stage model for hepatocarcinogenesis in rats to evaluate the influence of ovarian hormones on liver tumor promotion by TCDD. Our experimental design included quantitation of preneoplastic foci, cell proliferation, TCDD liver concentrations and cytochrome P-450d.

METHODS

Sprague-Dawley rats were ovariectomized (OVX) or sham-operated at 56 days of age. DEN (initiating agent) was administered i.p. in saline at a dose of 200 mg/kg at 70 days of age. Beginning at 77 days of age, rats were administered TCDD orally in corn oil once

every two weeks at a dose equivalent to 100 ng/kg/day. Intact and OVX rats each had four groups of rats; S/C (controls receiving saline and corn oil), S/TCDD (promoted only), DEN/C (initiated only) and DEN/TCDD (initiated and promoted). There were nine rats per group. Rats were sacrificed at 71 weeks of age (30 weeks of promotion) and livers removed.

Histological evaluation was conducted according to guidelines of the National Toxicology Program (8). Gamma glutamyltranspepidase (GGT) positive foci were detected as described previously (9) and positive staining indicated the presence of preneoplastic cells. Preneoplastic cells were also detected using a stain for placental glutathione transferase (PGT) (10). Preneoplastic foci were quantified in two ways; number of foci per cm^3 and proportion of liver occupied by foci using stereological analysis (11). In the cell proliferation studies, osmotic pumps filled with 20 mg/ml bromodeoxyuridine (BRDU) were implanted seven days prior to sacrifice. Immunohistochemical methods allowed for detection of BRDU-labelled hepatocyte nuclei undergoing replicative DNA synthesis. Non-lesioned hepatocytes (1000 cells per rat) were scored and the labelling index calculated. Liver TCDD concentrations were quantified as described previously (12) using GC-MS. Cytochrome P-450d concentrations were quantified by radioimmunoassay and these values were verified by analysis of P-450d protein on Western blots (6).

RESULTS AND DISCUSSION

In intact female rats, GGT positive foci were increased significantly in the DEN/TCDD livers. There were 44.7 foci per cm^3 in DEN/C rats compared to 387.5 in the DEN/TCDD group (Table 1). This finding was consistent with data on the percentage of liver occupied by GGT positive foci (data not shown). In contrast, only small increases in these parameters were evident in OVX rats. Chemically-induced increases in number and size of enzyme-altered foci in rat liver are generally considered as a reliable predictor of hepatocarcinogenic potential of selected chemicals (13, 14). In our studies, we have shown that the presence of the ovaries produces a dramatic sensitization of the rat liver to TCDD - mediated increases in GGT positive foci. *Similar effects were observed when PGT* positive foci were used to quantify enzyme-altered foci (data not shown).

There is increasing evidence that cell proliferation is important to the

carcinogenic actions of some chemicals (15). Cell proliferation appears to be especially relevant for evaluation of those chemicals which are non-genotoxic carcinogens. In our studies we observed that TCDD produced large increases in cell proliferation in intact females whereas no effect was detected in OVX animals (Table 1). The increase was evident in both S/TCDD and DEN/TCDD groups. These findings

Table 1 Summary of effects produced in a two stage model for hepatocarcinogenesis in intact OVX Rats

	S/C	S/TCDD	DEN/C	DEN/TCDD
GGT + foci/cm^3				
Intact	5.6	5.0	44.7	387.5[a]
OVX	0	0	30.4	80.7
BRDU Labelling Index				
Intact	0.3[a]	6.0[a]	0.8	7.3[a]
OVX	1.1	1.0	1.1	0.7
TCDD concentration(PPB)				
Intact	-	16.3[a]	-	18.3[a]
OVX	-	34.8	-	34.0
Cytochrome P-450d				
Intact	29	256	51	320
OVX	40	403	52	460

[a]significantly different from OVX at least at $p < 0.01$.
$n = 9$

suggest that TCDD, in the presence of ovarian hormones (probably estrogens), induces cell proliferation which increases the probability of clonal expansion of genetically-altered hepatocytes. Therefore, the preneoplastic foci and cell turnover data are consistent.

OVX rats have more adipose tissue than intact rats so one possible explanation for our findings was that more TCDD was present in livers of intact rats. However, we found that liver TCDD concentrations were twice as great in OVX rats. Therefore, ovarian-mediated sensitivity to the hepatocarcinogenic actions of TCDD are underestimated in our studies. Cytochrome P-450d is induced in rat liver but not extrahepatic tissues and this effect is dependent on the Ah receptor (6). P-450d is especially effective in catalyzing catechol estrogen formation from 17β-estradiol (6). This reaction is considered a possible mechanism for the metabolic activation of estrogens to DNA-damaging metabolites

(16). Our finding of a 10-fold induction of P-450d is consistent with the idea that TCDD may shift hepatic estrogen metabolism to DNA-damaging pathways.

In summary our data reveal that TCDD-estrogen interactions may play a dominant role in the hepatocarcinogenicity of TCDD.

REFERENCES

1. Kociba, R.J., Keyes, D.G., Beyer, J.E., Carreon, R.M. Wade, C.E., Dittenber, D.A., Kalnins, R.P., Frauson, L.E., Park, C.N., Barnard, S.D., Hummel, R.A., and Humiston, C.G. Toxicol. Appl. Pharmacol. 46:270-303 (1978).

2. National Cancer Institute. Carcinogenesis Tech. Rept. Ser. 122, 104PP., (1977)

3. Poland, A., and Glover, E. Cancer Res. 39:3341-3344 (1979).

4. Poland, A., Palen, D., and Glover, E. Nature 300(5889):271-273 (1982b).

5. Pitot, H.C., Goldsworthy, T., Campbell, H.A., and Poland, A. Cancer Res. 40:3616-3620 (1980).

6. Graham, M.J., Lucier, G.W., Linko, P., Maronpot, R.R., and Goldstein, J.A. Carcinogenesis 9:1935-1941 (1988).

7. Poland, A., and Knutson, J.C. Ann. Rev. Pharmacol. Toxicol. 22:517-554 (1982).

8. Maronpot, R.R., Montgomery, C.A.J., Boorman, G.A., and McConnell, E.E. Toxicol. Pathol 142:263-273 (1986).

9. Campen, D.B., Sloop, T.C., Maronpot, R.R., and Lucier, G.W. Cancer Res. 47:2328-2333 (1987).

10. Ito, N., Tatematsu, Hasegawa, R., and Tsuda, H. Toxicol. Pathol. 17:630-642 (1989).

11. Campbell, H.A., Pitot, H.C., Potter, V.R., and Laishes, B.A. Cancer Res. 42:465-472 (1982).

12. Sloop, T.C. and Lucier, G.W. Toxicol. and Applied Pharmacol. 88:329-337 (1987).

13. Popp, J.A., and Goldsworthy, T.L. Toxicol. Pathology 17:561-568 (1989).

14. Pitot, H.C., Campbell, H.A., Maronpot, R., Bawa, N., Rizvi, T.A., Xu, Y., Sargent, L., Dragan, Y., and Pyron, M. Toxicol. Pathol. 17:594-612 (1989).

15. Swenberg, J.A., Richardson, F.C., Bourcheron, J.A., Deal, F.H., Belinsky, S.A., Charbonneau, M., and Short, B.G. Environ. Health Perspectives 76:57-63 (1987)

16. Li, S.A., Klicka, J.K., and Li, J.J. Cancer Res. 45:181-185 (1985).