Influence of ovariectomy and 17 β-estradiol on the promotion of altered hepatocellular foci by TCDD

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Introduction

Chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induces liver tumors in female rats but not in male rats [1,2]. Although the mechanism for TCDD-induced hepatocarcinogenesis in female rats is currently unknown, several lines of evidence support a role for ovarian hormones. In two-stage initiation-promotion models, hepatic tumor incidence is lower in TCDD-treated ovariectomized (OVX) female Sprague-Dawley rats than in TCDD-treated sham operated rats [3]. The formation of 8-hydroxydeoxyguanosine adducts, a marker for oxidative DNA damage, is lower in hepatic nuclear DNA of TCDD-treated ovariectomized than TCDD-treated intact female Sprague-Dawley rats [4]. The induction of cell proliferation by TCDD is dependent upon ovarian hormones [5]. The number and percentage of the liver occupied by gamma-glutamyltranspeptidase (GGT) enzyme-altered hepatocellular foci are significantly lower in TCDD-treated ovariectomized rats than intact rats [5]. The number and volume fraction of foci expressing the placental form of glutathione-S-transferase (PGST) is lower in TCDD-treated ovariectomized rats compared to intact rats [5].

It is hypothesized that estrogen, specifically, is involved in the mechanism of TCDD-induced hepatocarcinogenesis by the enzyme-mediated metabolic activation of estradiol to direct or indirect acting reactive intermediates [6] or through alteration of cell growth pathways [7]. TCDD induces cytochrome P450 isozymes capable of estradiol metabolism to catechol estrogens [8] which demonstrate carcinogenicity in the Syrian hamster model of estrogen-induced carcinogenesis [9]. Down regulation of the epidermal growth factor receptor by TCDD is not observed in ovariectomized animals suggesting that this effect may be involved in the mechanism of induction of cell proliferation by TCDD [7]. Consequently, TCDD may be acting through multiple mechanisms involving both increased oxidative damage and altered cell growth pathways contributing to the formation of development of altered hepatocellular foci.

The aim of the current study was to test the hypothesis that estrogen is involved in the induction of PGST– and GGT-positive enzyme-altered hepatocellular foci following chronic exposure to TCDD. If this hypothesis is correct, we may expect that co-treatment of ovariectomized female rats with both TCDD and 17 β-estradiol will result in the increased formation and growth of altered hepatic foci in a tumor promotion model. To test this hypothesis DEN-initiated and ovariectomized female rats were treated with TCDD for 30 weeks in the presence and absence of estrogen which was administered continuously by implanted 90-day release pellets.

Materials and Methods

Animals were housed three per cage under conditions of controlled temperature (70 ± 0.5°F), humidity (50 ± 5%), and lighting (12 hour light/12 hour dark), and received food and water *ad libitum*. Animals were ovariectomized or sham operated at 8 weeks of age. Animals were initiated with 175 mg diethylnitrosamine/kg at 10 weeks of age. One week after initiation, ovariectomized animals were implanted with 90-day release pellets containing 0 mg (placebo) or 0.17mg of 17 *β*-estradiol/pellet (Innovative Research). Intact animal received placebo pellets only. New pellets were implanted after 90 days. Starting one week later, animals were treated weekly with 700 ng TCDD/kg or corn oil vehicle for 30 weeks. (This dose of TCDD is equivalent to an averaged daily dose of 100 ng TCDD/kg/day). Osmotic pumps (Alzet Corp.) containing 30 mg/ml 5-bromo-2'-deoxyuridine in saline, were implanted subcutaneously seven days prior to necropsy to allow for evaluation of TCDD-induced changes in cell proliferation. Animals were killed by asphyxiation with CO_2, tissues removed, weighed, sectioned and fixed in 4% paraformaldehyde or frozen in liquid nitrogen. Fixed sections were paraffin embedded and liver sections (5 microns thick) were cut and placed on microscope slides. PGST immunohistochemistry and GGT enzyme-histochemistry were performed as previously described [5]. PGST- and GGT-positive altered hepatocellular foci analyses were performed on a Macintosh computer using the public domain NIH Image program (U.S. National Institutes of Health) and subjected to stereological analysis as described previously [5]. Differences between groups were analyzed by Kruskal-Wallis and Mann-Whitney U test, $p < 0.05$.

Results and Discussion

Effect of TCDD in intact and OVX rats - In intact rats, the number and volume fraction of both GGT-positive foci (Table 1) and PGST-positive foci (Table 2) were higher in TCDD-treated rats compared to control rats. In ovariectomized rats, the number and volume fraction of PGST-positive foci were also higher in TCDD-treated rats compared to control rats (Table 2). These data demonstrate that TCDD is a promoter of altered hepatocellular foci in the livers of both intact and OVX rats. These findings are consistent with a previous study [5]. However, in that study increases were observed in the formation of GGT-positive foci in TCDD-treated OVX rats. In the present study, no significant differences in the number of foci or the volume fraction were observed between TCDD-treated and control rats. However, the number of foci/cm^3 observed in control OVX rats in this study was nearly three-fold higher than previously observed.

Effect of ovariectomy and TCDD – The number and volume fraction of GGT-positive foci was higher in TCDD-treated intact rats than in TCDD-treated OVX rats (Table 1). These values were not statistically significant. The observations of higher levels of GGT foci in TCDD treated intact rats is consistent with those observed in the previous study. No statistically significant differences were observed in the present study or previously in the number of PGST-positive foci/cm^3 or volume fraction between TCDD-treated intact or OVX rats.

Effect of supplemental 17 β-estradiol and TCDD - The number of GGT-positive foci/cm^3 were significantly higher in rats co-treated with TCDD and 17 *β*-estradiol compared to those receiving 17 *β*-estradiol alone (Table 1). However, the number of GGT-positive foci/cm^3 in these rats was

considerably lower than in rats not receiving 17 β-estradiol. For example, the mean number of foci/cm^3 in control rats receiving 17 β-estradiol alone was 3.0 compared to 28.0 in intact controls and 119.9 in OVX controls. Likewise, the mean number of foci/cm^3 in TCDD-treated rats receiving 17 β-estradiol was 33.5 compared to 177.7 in TCDD-treated intact and 96.3 in TCDD-treated OVX rats. No significant differences were observed in volume fractions between treatments. These data in combination with the observed effects in OVX rats suggest an influence of estrogen on GGT-positive foci formation. The overall number of GGT-positive foci was decreased in rats exposed to 17 β-estradiol. However, TCDD-induced increases in GGT-positive foci formation were observed in both OVX rats receiving supplemental 17 β-estradiol and intact rats with functional estrous cycles. The number and volume fraction of PGST-positive foci were not significantly different in 17 β–estradiol treated-rats as compared to OVX rats not receiving 17 β–estradiol.

Although TCDD is a promoter of enzyme-altered hepatocellular foci in both intact and OVX rats, only GGT-positive foci were sensitive to the inhibitory effect of ovariectomy. These data suggest that the formation of PGST- and GGT-positive foci were not proportionate. The overall formation of GGT-positive foci was decreased by 17 β-estradiol, but TCDD-induced formation of GGT-positive foci was only observed in 17 β-estradiol supplemented OVX rats and normal estrous-cycling rats. These data suggest that endogenous and exogenous estrogens both positively and negatively influence hepatic tumor promotion by TCDD. The dependency of effects on foci phenotype demonstrates the importance of determining the critical link between foci phenotype and tumor formation in tumor promotion studies.

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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.* **1978**, **46**, 279-303.
2. N.T.P. (1982) Bioassay of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin for possible carcinogenicity (gavage study). NTP, Research Triangle Park, NC.
3. Clark, G., Tritscher, A., Maronpot, R., Foley, J. and Lucier, G. (1991) Tumor promotion by TCDD in female rats. In Gallo, M.A., Scheuplein, R.J., and Van Der Heijden, K.A. (ed.), *Banbury Report 35: Biological Basis for Risk Assessment of Dioxins and Related Compounds*. Cold Springs Harbor Laboratory Press.
4. Tritscher, A.M., Seacat, A.M., Yager, J.D., Groopman, J.D., Miller, B.D., Bell, D., Sutter, T.R. and Lucier, G.W.; *Cancer Lett.* **1996**, 98, 219-225.
5. Lucier, G.W., Tritscher, A.M., Goldsworthy, T., Foley, J., Clark, G., Goldstein, J. and Maronpot, R.; *Cancer Res.* **1991**, **51**, 1391-1397.
6. Yager, J.D. and Liehr, J.G.; *Annu. Rev. Pharmacol. Toxicol.* **1996**, 36, 203-232.

7. Sewell, C.H., Lucier, G.W., Tritscher, A.M. and Clark, G.C.; *Carcinogenesis* **1993**, 14, 1885-1893.
8. Graham, M.J., Lucier, G.W., Linko, P., Maronpot, R.R. and Goldstein, J.A.; *Carcinogenesis* **1988**, 8, 1935-1941.
9. Liehr, J.G., Fang, W.F., Sirbasku, D.A. and Ari-Ulubelen, A.; *J. Steroid Biochem.* **1986**, 24, 353-356.

Table 1. Putative preneoplastic GGT-positive foci following DEN initiation and 30 weeks of TCDD promotion

Dosing Group		Estrogen (mg/pellet)	Lesions/cm3	Volume Fraction
Intact [a]	Control	0	28.0 ± 19.3	0.01 ± 0.02
Intact [a]	TCDD	0	177.7 ± 263.9	0.22 ± 0.34 [c]
OVX [a]	Control	0	119.9 ± 110.8	0.11 ± 0.13
OVX [b]	TCDD	0	96.3 ± 75.6	0.17 ± 0.13
OVX [b]	Control	0.18	3.0 ± 7.7	0.05 ± 0.13
OVX [b]	TCDD	0.18	33.5 ± 28.5 [c]	0.03 ± 0.03

[a] Mean ± Standard deviation, nine animals per group.
[b] Mean ± Standard deviation, eight animals per group
[c] Significantly different from corresponding estrogen corn oil control

Table 2. Putative preneoplastic PGST-positive foci following DEN initiation and 30 weeks of TCDD promotion

Dosing Group		Estrogen (mg/pellet)	Lesions/cm3	Volume Fraction
Intact [a]	Control	0	361.8 ± 200.7	0.30 ± 0.19
Intact [a]	TCDD	0	978.7 ± 656.6 [c]	1.23 ± 0.88 [c]
OVX [a]	Control	0	133.0 ± 52.2	0.26 ± 0.14
OVX [b]	TCDD	0	873.1 ± 754.5 [c]	0.97 ± 0.86 [c]
OVX [b]	Control	0.18	193.5 ± 163.2	0.14 ± 0.14
OVX [b]	TCDD	0.18	691.2 ± 403.5 [c]	0.75 ± 0.48 [c]

[a] Mean ± Standard deviation, nine animals per group.
[b] Mean ± Standard deviation, eight animals per group
[c] Significantly different from corresponding estrogen corn oil control