Role of estrogen in the mechanism of hepatocarcinogenesis induced by TCDD in female rat liver.

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Introduction

Liver tumor incidence in the TCDD-treated female Sprague-Dawley rats is widely used by regulatory agencies around the world for setting guidelines for human exposure to TCDD and dioxin-like compounds. Currently, the mechanism for the induction of liver tumors by TCDD in the female rats is unknown. Therefore a better understanding of the how TCDD induces liver cancer in rodents and the relevance of this mechanism to human populations will reduce uncertainty in risk assessment for human exposure to dioxins.

Chronic exposure to TCDD induces liver tumors in female but not male rats (1). In two-stage initiation promotion models, the induction of cell proliferation by TCDD is dependent upon ovarian hormones and the induction of enzyme-altered hepatic foci is modulated by ovariectomy (OVX) (2,3) (Table 1). These observations suggest a role for ovarian hormones, presumably estrogen, in the mechanism of hepatocarcinogenesis induced by TCDD.

Table 1. Evidence for a role for ovarian hormones in the hepatocarcinogenic mechanism of TCDD in female rat liver.

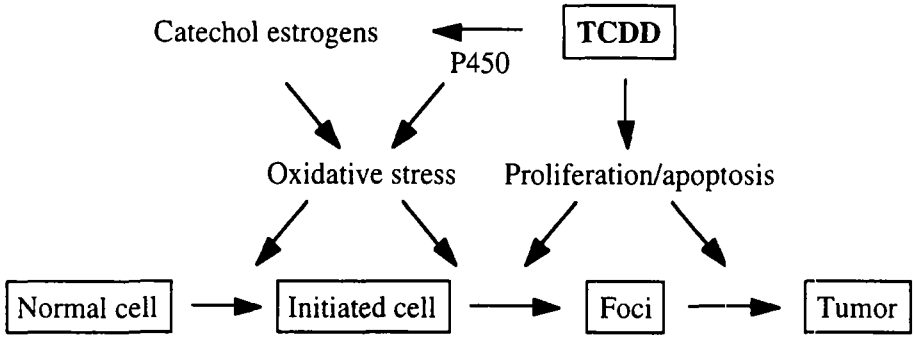
Groups	BrdU Labeling Index (%)		GGT foci vol fraction $x10^{-2}$		8-OH-dG/10^6 dG	
	Intact	OVX	Intact	OVX	Intact	OVX
S/C	0.3 ± 0.1	1.1 ± 0.2	1 ± 1	0 ± 0	13 ± 4	9 ± 3
S/T	6.0 ± 4.0	1.0 ± 0.2	1 ± 1	0 ± 0	65 ± 100	11 ± 4
D/C	0.8 ± 0.1	1.2 ± 0.4	3 ± 1	3 ± 1	12 ± 4	6 ± 1
D/T	7.3 ± 3.1	0.7 ± 0.3	37 ± 6	8 ± 4	31 ± 19	9 ± 4

S, saline-non initiated; D, DEN initiated; C, corn oil controls; T, 100 ngTCDD/kg/d for 30 weeks

It has been hypothesized that induction of estradiol metabolism by TCDD-inducible cytochrome P450 results in oxidative stress resulting in DNA damage and indirect genotoxicity (6). TCDD induces cytochrome P450 isozymes that metabolize estradiol to catechol estrogens (5). In the Syrian hamster model of estrogen-induced carcinogenesis, the catechol estrogen 4-hydroxyestradiol is as carcinogenic as 17β-estradiol (4). Catechol estrogens are potential sources of reactive oxygen species through redox cycling of

semiquinone and quinone intermediates. This hypothesis is supported by observations that oxidative DNA damage is higher in intact rats chronically exposed to TCDD compared with ovariectomized rats (7) (Table 1). 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 (8). Consequently, TCDD may be acting through multiple mechanisms involving both increased oxidative damage and altered cell growth pathways (Figure 1).

Figure 1. Potential mechanisms of hepatocarcinogenesis of TCDD.



The effect of lower estrogen levels after ovariectomy may be to decrease the sensitivity of the liver to TCDD by simply shifting the dose-response curve for TCDD. Alternatively endogenous promotion by estrogen may be synergistic with promotion by TCDD, which is lost after ovariectomy.

The aim of this current study was to test the hypothesis that estrogen is involved in the induction of hepatocarcinogenesis following chronic exposure to TCDD. If this hypothesis is correct, we may expect that co-treatment of ovariectomized female rats with both TCDD and 17β-estradiol will result in increased cell proliferation and altered hepatic foci formation in a tumor promotion models. In addition if we may predict that there may be an increase in oxidative damage as a result of estrogen metabolites. To test this hypothesis DEN-initiated and ovariectomized female rats were treated with TCDD for 20 weeks and 30 weeks, in the presence of various dose of estrogen, administered continuously by implanted 90-day release pellets.

Materials and Methods

Animals were housed three to a 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) 0.17mg (low), 0.34mg (medium) or 1.8 mg (high) of 17β-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 20 weeks. (This dose of TCDD is equivalent to an averaged daily dose of 100 ngTCDD/kg/day which is equivalent to the highest dose used in the 2-year cancer bioassay of Kociba et al.(1)). Osmotic pumps (Alzet model 2ML1;

10µl/hr delivery rate; Alzet Corp., Palo Alto, CA) 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. Serum was obtained from cardiac blood obtained under CO_2, anesthesia. Animals were killed by asphyxiation with CO_2, tissues removed, weighed, sectioned and fixed in paraformaldehyde or frozen. Significant differences in serum estradiol levels were tested by ANOVA and Fisher's PLSD test $P<0.05$. Differences in body weights and tissue weights were analyzed by GLM.

Results and Discussion

Ovariectomy resulted in a higher body weights compared with intact rats. Treatment with estrogen resulted a transient decrease in body weight, followed by increased bodyweight gain. After 20 weeks, the body weights in estrogen-treated animals was similar to those of intact rats. As observed in previous studies, chronic exposure to TCDD resulted in a decreased body weight gain in intact rats (Table 2). The TCDD-induced decrease in body weight gain was also observed in ovariectomized animals co-treated with TCDD and the low and mid-dose-estrogen pellets. However there was no reduction in body weight gain in the TCDD treated animals treated with the highest dose estrogen pellet. TCDD caused a significant increase in relative liver weight (Table 2).

In order to assess the release of estrogen from the pellets we periodically measured the serum levels of 17β-estradiol in three additional animals from each group in a parallel study. Two weeks after implantation, serum levels were > 200pg/ml, and proceeded to decrease over time to <40 pg/ml, 12 weeks after implantation in the TCDD-treated groups. The decrease may have been due to a decreased dose/weight ratio as the animal body weights increased with time and/or that the estrogen release from the pellets was not constant. Serum estradiol levels were measured in all animals at the end of the 20-week study. As expected, ovariectomy caused a significant reduction in serum estrogen levels compared with intact cycling rats (Table 2).The levels in the intact rats was consistent with the range of 17β-estradiol levels in normal female rats (mean level 40 ng/ml in cycling rats). An increase in serum levels as a result of the implantation of the pellets was observed for all three doses of 17β-estradiol pellets. Terminal serum levels in animals receiving 17β-estradiol pellets were in the physiological range and were significantly higher at necropsy than in rats implanted with placebo pellets (Table 2). There was no significant effect of TCDD treatment on serum 17β-estradiol levels in any group at this time point.

Previous studies have demonstrated an antiestrogenic effect of TCDD both *in vivo* and *in vitro*. The *in vivo* effectiveness of the estrogen pellets and the effect of TCDD on these responses was assessed by measurement of the uterine wet-weight in the animals at necropsy (Table 2). As expected the reduction in serum estrogen level as result of ovariectomy led to a significant reduction in uterine wet weight. The increase in 17β-estradiol levels as a result of pellet implantation led to a dose-dependent increase in uterine wet weight. There was no significant effect of TCDD treatment on uterine wet weight or relative uterine wet weight in intact cycling rats. Furthermore there was no effect of TCDD on the estrogen-induced increase in uterine wet weight in ovariectomized rats indicating that within this experimental framework, TCDD did not exhibit an antiestrogenic effect on the uterus. This is in contrast to the antiestrogenic effect of TCDD on estrogen-induced uterine wet weight gain in mice.

These data indicated that chronic co-treatment of estrogen and TCDD did not result in any marked toxicity. The *in vivo* efficacy of the estrogen treatment and the lack of an antiestrogenic effect of TCDD on either serum estradiol levels or uterine wet weight indicate that this experimental model is suitable for the analysis of the effect of estrogen on liver tumor promotion by TCDD. Ongoing analyses are currently investigating the effect of the co-treatment with estrogen on the induction of cell proliferation and enzyme altered liver foci formation by TCDD in the livers of animals.

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Table 2. Effect of TCDD and estrogen on body weights, tissue weights and serum estradiol.

Dosing Group		Estrogen (mg/pellet)	Body wt (g)	liver weight (g/100g B.wt)	serum estradiol (pg/ml)	uterine weight (g/100g B.wt.)
Intact	Control	0	341 ± 24	3.7 ± 0.4	41 ± 33	0.21 ± 0.07
Intact	TCDD	0	315 ± 30	3.9 ± 0.3	27 ± 11	0.19 ± 0.05
OVX	Control	0	472 ± 92	2.6 ± 0.2	12 ± 4	0.02 ± 0.00
OVX	TCDD	0	398 ± 32	3.4 ± 0.3	11 ± 5	0.03 ± 0.01
OVX	Control	0.17	366 ± 51	3.4 ± 0.2	37 ± 10	0.16 ± 0.04
OVX	TCDD	0.17	322 ± 41	4.2 ± 0.5	49 ± 13	0.21 ± 0.03
OVX	Control	0.34	348 ± 43	3.5 ± 0.4	48 ± 47	0.19 ± 0.07
OVX	TCDD	0.34	307 ± 36	4.2 ± 0.4	57 ± 43	0.24 ± 0.11
OVX	Control	1.8	332 ± 27	3.5 ± 0.2	63 ± 27	0.23 ± 0.05
OVX	TCDD	1.8	340 ± 48	3.9 ± 0.2	68 ± 53	0.25 ± 0.14

Values represent the mean ± standard deviation for nine animals per group.