Effect of TCDD on the Transient Suppression of Estrogen-Dependent MCF-7 Human Breast Tumor Growth *In Vivo*

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Introduction

Various studies have shown that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin TCDD exhibits a broad spectrum of adverse responses which are tissue and species specific (1). It has been suggested that many of the toxic effects of TCDD in animals, such as depressed fertility, fetotoxicity, teratogenicity, immunosuppression, and the wasting syndrome could be linked to endocrine disruption of estrogenic activity (2). This is supported by the observed antiestrogenic activity associated with TCDD exposure in the rodent uterotropic assay and suppression of various estrogen responsive effects in MCF-7 human breast cancer cells (1). Of particular interest was the ability of low doses of TCDD to suppress the development of spontaneous, age related mammary tumors (3) and carcinogen induced mammary tumors in rats (4) and inhibition of estrogen dependent postconfluent cell proliferation leading to formation of multicellular nodules or foci in MCF-7 cultures (5).

In an effort to further elucidate the antiestrogenic action of TCDD, we previously examined the effects of TCDD exposure on estrogen dependent MCF-7 tumor xenograft growth in BDF_1 mice. We reported that concurrent exposure to 5 μg TCDD per kg body weight resulted in suppression of 17β-estradiol (E_2)-dependent tumor growth for two weeks with a loss of suppression during the third week (6). Studies have continued to further characterize and determine the mechanistic basis of this transient suppression of an estrogenic response in this surrogate human target organ. Results of these studies suggest that the antiestrogenic response is host mediated since pretreatment of mice with TCDD for two weeks results in the lack of subsequent suppression of E_2 dependent MCF-7 tumor growth by concurrent TCDD exposure.

Materials and Methods

Unless otherwise indicated, details of the MCF-7 cell culture conditions, xenograft implantation and measurement, and treatment protocols are as previously described (6). Briefly, 2.0 X 10^6 MCF-7 cells, congealed in a fibrin clot, were implanted under the kidney capsule of male B6D2F_1 mice supplemented with E_2 pellets s.c. and immunosuppressed by daily treatment with cyclosporin A (60mg/kg s.c.). Two perpendicular diameters of the tumor xenografts were measured *in situ* and tumor volumes were calculated once a week during survival laparotomy. TCDD in corn oil was injected i.p. once a week at the schedules indicated.

Results and Discussion

Previous studies used multiple i.m. injections of E_2 every four days to maintain circulating E_2 levels. Examination of serum E_2 concentration by radioimmuno assay showed fluctuations from 0.2 to 1.0 ng per ml (data not shown). Silastic implants containing E_2 were used to stabilize serum E_2 levels and parameters were chosen to provide three week tumor growth midway on the linear portion of the E_2 dose response curve which is equivalent to the response obtained with 50 μg E_2 doses used in the previous studies (data not shown).

A dose of 5 μg TCDD per kg body weight every 8 days to MCF-7 xenograft mice supplemented with E_2 silastic implants resulted in the same two week transient suppression of tumor growth observed in previous studies using multiple E_2 injections (Figure 1). E_2-dependent tumor growth was validated by the finding that there was no tumor growth observed in the corn oil control group not supplemented with E_2 implants.

To distinguish between host and tumor related effects resulting in the observed TCDD suppression of E_2 induced tumor growth, mice were pre-treated with TCDD (5 μg/ kg body weight) for two weeks prior to tumor implantation and E_2 supplementation. The results shown in Figure 1. indicate that pretreatment with TCDD eradicates the TCDD suppressive effects of E_2 stimulated tumor growth as seen in concurrent experiments where TCDD treatment was begun at the time of tumor implantation. These results suggest that the interaction between the mouse and TCDD during the two week pretreatment may have made the mouse refractory to the antiestrogenic effects of this particular, and otherwise effective, TCDD treatment.

It is possible that during the two week pretreatment, TCDD metabolism had occurred and its metabolites had accumulated. It is known that certain hydroxylated PCBs exhibit estrogen receptor binding and have estrogenic activity (7). It is possible that hydroxylated TCDD metabolites could also be estrogenic and that this could lead to the observed estrogen dependent tumor growth which may not be dependent directly on the E_2 supplementation. To examine this possibility, mice were exposed to TCDD without E_2 supplementation using both the two week pre-implant exposure followed by three week post implant exposure as well as the three week post-implant exposure. The results showed no increase in tumor growth at any time compared to the usual E_2-dependent growth and transient TCDD suppressed tumor growth (data not shown). This indicates that the observed tumor growth after two weeks of TCDD treatment, either pre- or post-implantation, is not due to the TCDD itself or putative TCDD metabolites but to E_2.

It is possible that the MCF-7 tumor has changed during the second to third week of

TCDD treatment. This could contribute to tumor growth due either to loss of E_2 dependency or loss of TCDD sensitivity. To examine this possibility, tumors from mice treated with TCDD for three weeks and exhibiting TCDD resistant, E_2-dependent growth were transplanted into naive mice followed by the usual E_2 supplementation and TCDD treatment. As seen in Figure 2., the tumor again demonstrated E_2 growth dependency as well as the two week transient TCDD suppression of growth observed in the original implantation experiments. These results indicate that the MCF7 tumor itself has not changed in this regard as a result of the previous TCDD treatment and are consistent with a TCDD mediated change in the mouse host rather than in the tumor.

The results of these experiments have extended our previous studies which demonstrated TCDD suppression of E_2-dependent MCF-7 tumor growth *in vivo* (6). It was hypothesized that this suppression was due to E_2 depletion in the tumor by the induction of cytochrome P450s 1A1 and 1B1 as was shown to occur in MCF-7 cultures (8). It was also considered that competition or cross-talk between the occupied estrogen and Ah receptors at the level of the estrogen response elements in the tumor could play a role in the apparent antiestrogenic suppression of tumor growth (9). Taken together, the results of this study do not support this hypothesis but rather point to a different, host mediated, mechanism. This mechanism appears to be transient in nature, and could possibly be associated with a relatively slow alteration of the host which diminishes the effective potency and/or systemic concentration of TCDD. Recent reports by Diliberto *et al.* (10) indicate the sequestering of TCDD from adipose tissue to the liver, possibly through an Ah receptor mediated induction of hepatic CYP1A2, and subsequent binding and effective inactivation of TCDD. The results described here suggest possible significant physiological ramifications of this sequestration in regard to risk assessment and drug resistance based on accommodation phenomena. Further studies involving the role of the Ah receptor competency and activation of specific host gene expression at the molecular level will further clarify this possibility.

Figure 1

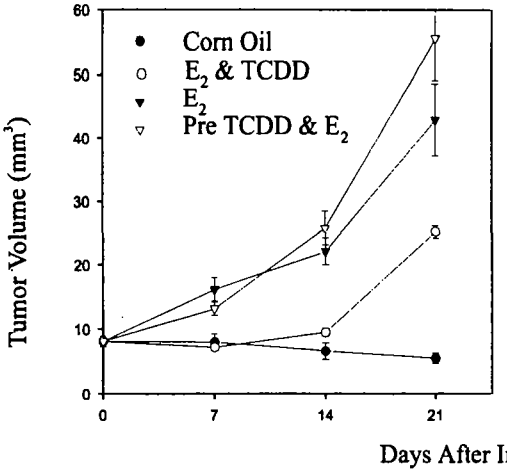
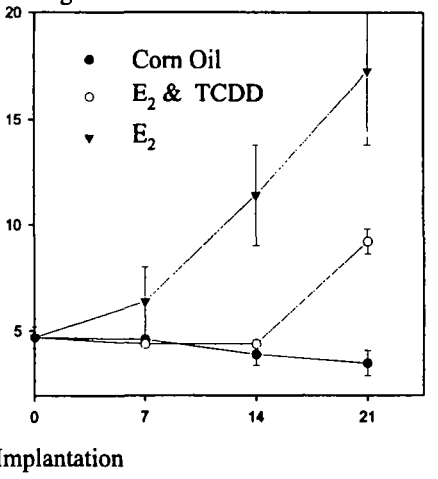


Figure 2



Days After Implantation

Acknowledgments

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Figure Legends

Figure 1. Effect of TCDD on MCF-7 E_2-dependent tumor growth with E_2 silastic implants. E_2 silastic implants were inserted into xenograft bearing mice at the time of tumor implantation. Mice were treated i.p. with TCDD (5 μg /kg) in corn oil on days 14 and 7 pre-implantation and 1, 8 and 15 after implantation or only on days 1, 8 and 15 after implantation. Tumor volume was determined at the indicated times. Error bars are standard error of the mean of 5 mice per group of a representative experiment.

Figure 2. Transplanted MCF-7 tumor growth sensitivity to E_2 and TCDD. MCF-7 tumors from E_2-TCDD treated mice showing loss of TCDD suppression of E_2-dependent growth at three weeks as in Figure 1 were transplanted in untreated mice which were then treated as in the Figure 1 post implantation protocol.

References

1. Safe, S; *Annu. Rev. Pharmacol. Tox.* **1986,** 26, 371.
2. Umbreit, T and Gallo, M; *Tox. Letts.* **1988,** 42, 5.
3. Kociba, R, Keyes, D, Beyer, J, Carreon, R, Wade, C, Dittenber, D, Kalnins, R, Frauson, L, Park, C, Barnard, S, Hummel, R and Humiston, C; *Tox. App. Pharm.* **1978,** 46, 279.
4. Holcomb, M and Safe, S; *Can. Letts.* **1994,** 82(1), 43.
5. Gierthy, J, Lincoln II. D, Roth, K, Bowser, S, Bennett, J, Bradley, L and Dickerman, H; *J. Cell. Biochem.* **1991,** 45, 177.
6. Gierthy, J, Bennett, J, Bradley, L and Cutler, D; *Can. Res.* **1993,** 53, 3149.
7. Gierthy, J, Arcaro, K and Floyd, M;. *Chemos.* **1997,** 34,1495.
8. Spink, D, Lincoln II, D, Dickerman, H and Gierthy, J; *Proc. Natl. Acad. Sci.* **1990,** 87, 6917.
9. Safe, S; *Pharm. & Therap.* , 67(2), 247.
10. Diliberto, J, Burgin, D, and Birnbaum, L; *Biochem. & Biophys. Res. Com.* **1997,** 236(2), 431.