Inhibition of TCDD-Induced Responses in B6C3F1 Mice And Hepa 1c1c7 Cells by Indole-3-Carbinol

I. Chen, N. Harper and S. Safe
Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX 77843

1. Introduction

Indole-3-carbinol (I3C) and related heterocyclic compounds occur widely in a number of vegetables such as cabbage, cauliflower and Brussels sprouts. Several studies have reported that I3C and several acid-derived condensation products bind to the aryl hydrocarbon receptor (AhR) and induce several AhR-mediated responses including the induction of CYP1A1 and CYP1A2 gene expression in laboratory animals and mammalian cells in culture [1-4]. I3C and a more potent analog, indolo[3,2-*b*]carbazole (ICZ) also exhibit antiestrogenic activity in MCF-7 human breast cancer cell lines [5,6]. Both I3C and TCDD inhibit mammary tumor formation in rodents [7-9] and it has been suggested that these effects may be related to induction of CYP1A2-dependent estradiol-2-hydroxylase activity and the subsequent metabolic inactivation of endogenous 17β-estradiol [5,8]. However, there is also evidence that the antiestrogenic activity of I3C is caused via AhR-mediated responses which are independent of estrogen metabolism [6]. I3C binds with low affinity to the AhR and therefore it is possible that this weak AhR agonist may exhibit partial AhR antagonist activity. This study reports the interaction of I3C and TCDD in mouse Hepa 1c1c7 hepatoma cells and in B6C3F1 mice. The results of this study demonstrate that I3C inhibits two TCDD-induced responses, namely, immunotoxicity and induction of Cyp1a-1 gene expression.

2. Materials and Methods

Chemicals and Biochemicals. TCDD, [^{3}H]TCDD (32 Ci/mmol), 2,3,7,8-tetrachlorodibenzofuran (TCDF), and ethoxyresorufin were prepared in this laboratory. I3C was purchased from Sigma Chemical Company (St. Louis, MO). Guinea pig complement, RPMI 1640 media and Earls Balanced Salt Solution (EBSS) were purchased from GIBCO Laboratories (New York, NY). Trinitrophenyl-lipopolysaccharide (TNP-LPS, Sigma #T4020), picryl sulfonic acid and glycyl-glycine, goat anti-mouse IgM conjugated to alkaline phosphatase (IgM-AP, #A7784), and *p*-nitrophenyl phosphate (NPP, #104) were obtained from Sigma Chemical Co. All other chemicals used were of the highest grade commercially available.

Maintenance of Hepa 1c1c7 Cells. Wild-type Hepa 1c1c7 cells were kindly provided by Dr. J. P. Whitlock, Jr. (Stanford University). The cells were grown as a continuous cell line in α-MEM supplemented with 2.2 mg/ml tissue culture grade sodium bicarbonate, 5% fetal calf serum (v/v) and 10 ml/l antibiotic/antimycotic solution. Stock cultures were grown in 150-cm^2

tissue culture flasks and incubated in a humidified mixture of air:carbon dioxide (95:5) under atmospheric pressure. After 24 hr, cells were harvested by trypsinization and treated for 2 hr at 37°C in a shaking water bath. Nuclear extracts were obtained from cells treated with 10 nM TCDD, 1 μM MCDF and 10 μM αNF.

Ethoxyresorufin O-Deethylase (EROD) Activity. At 70% confluency, cells were dosed with DMSO, 1 nM TCDD, different concentrations of I3C alone or in combination with 1 nM TCDD. After 24 hr, cells were harvested manually by scraping, resuspended in Tris sucrose buffer (38 mM Tris, 0.2 M sucrose, pH 8.0) and EROD activity was determined fluorimetrically[10].

Immunotoxicity Studies. B6C3F1 female mice were obtained from an in-house breeding colony at 3 weeks of age and were allowed to mature to 8 weeks of age before use. The mice (4 to 5 per group) were gavaged with a corn oil vehicle, TCDD, I3C, or TCDD + I3C in a total volume of 10 μL corn oil/g body weight. The mice were immunized i.p. with 50 μg TNP-LPS 24 hr later and sacrificed 72 hr after immunization. The mice were terminated by cervical dislocation and the spleens collected. A single cell suspension of spleen cells was prepared, washed, and resuspended in RPMI 1640 media. The Cunningham modification of the Jerne plaque forming cell (PFC) assay was used to assess humoral immune function [11,12]. The target sheep erythrocytes were haptenated according to the method of Rittenberg and Pratt [13].

3. Results and Discussion

The results presented in Figure 1 illustrate that 1 nM TCDD induced EROD activity in Hepa 1c1c7 cells. In contrast, I3C did not cause a significant induction response at concentrations from 1 to 500 μM. These data are consistent with the relatively low activity of

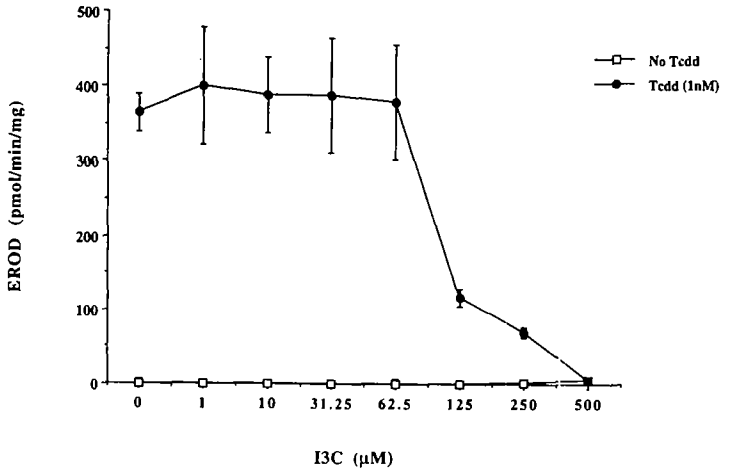

Figure 1. Induction of EROD activity in mouse Hepa 1c1c7 cells by I3C (□) alone and 1 nM TCDD plus different concentrations of I3C (●).

I3C as an AhR agonist [1-6]. However, in Hepa 1c1c7 cells cotreated with different concentrations of I3C plus 1 nM TCDD, there was a significant decrease in EROD activity at the higher concentrations of I3C (125 to 500 μM). Similar results have also been observed in other Ah-responsive human breast cancer cell lines and the inhibitory effects of I3C on CYP1A1 induction by TCDD are paralleled by decreased levels of the nuclear AhR complex and decreased Cyp1a-1 mRNA levels (data not shown).

In a parallel study, B6C3F1 mice were treated with the T cell-independent antigen TNP-LPS and TCDD significantly inhibited the PFC-response to this antigen (Table 1). In contrast, I3C alone caused only minimal inhibition of the antigen-induced splenic PFC response at doses as high as 50 mg/kg (data not shown). In B6C3F1 mice cotreated with I3C plus TCDD, there was a significant dose-dependent inhibition of TCDD-induced immunotoxicity by I3C and, at a dose of 5 mg/kg, the immunotoxic effects of TCDD in B6C3F1 mice were completely inhibited. These data show that an I3C/TCDD ratio of 1000/1 was sufficient for total inhibition of TCDD-induced immunotoxicity in B6C3F1 mice.

Table 1. Effects of TCDD, I3C and TCDD plus I3C on the PFC Response to TNP-LPS in B6C3F1 Mice.

Treatment (dose)	Cells/Spleen x 10^8	PFCs/Spleen x 10^5	PFCs/10^6 Cells
Control	1.71 ± 0.14	4.22 ± 0.31	2545 ± 314
TCDD (5 μg/kg)	1.47 ± 0.12	2.10 ± 0.09[a]	1485 ± 167[a]
TCDD (5 μg/kg) + I3C (1 mg/kg)	1.61 ± 0.24	2.31 ± 0.06[a]	1637 ± 361[a]
TCDD (5 μg/kg) + I3C (5 mg/kg)	1.60 ± 0.13	3.92 ± 0.30[b]	2508 ± 242[b]
TCDD (5 μg/kg) + I3C (10 mg/kg)	1.46 ± 0.07	4.43 ± 0.19[b]	3079 ± 233[b]

[a] *significantly lower ($p < 0.05$) than observed in control animals.*
[b] significantly higher ($p < 0.05$) than observed in TCDD-treated animals.

In most Western countries, the dietary intake of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) varies from 150 to 1500 pg/day and using the toxic equivalency factor approach, the estimated daily intake of PCDDs/PCDFs in terms of "dioxin" or toxic equivalents (TEQs) varies from 80 to 120 pg/day [14]. The adverse human health effects of background levels of PCDDs/PCDFs is unknown; however, any assessment of possible adverse effects of these compounds should take into account dietary intakes of other natural AhR agonists (or "endodioxins") such as I3C, polynuclear aromatic hydrocarbons and aromatic amines and their possible additive or non-additive interactions. I3C is a weak AhR agonist which undergoes extensive acid-catalyzed self-condensation reactions to form a large number of products including indolo[3,2-b]carbazole (ICZ), a moderately active AhR agonist [1]. Based on an estimated daily intake of 25 g of cruciferous vegetables and an I3C concentration of 5 μmol/g, the estimated intake of I3C is approximately 735,000,000 pg/day. Therefore, the dietary intake of mass balance ratio of I3C/(PCDDs + PCDFs) is > 49,000/1 and the corresponding I3C/TEQ ratio is > 612,000/1. The corresponding I3C/TCDD ratios required for inhibition of TCDD-induced immunotoxicity in B6C3F1 mice and CYP1A1 in mouse Hepa 1c1c7 cells was 1000/1 and 125,000/1 and these ratios are all lower than the corresponding ratio (< 612,000/1) for dietary intake of these compounds. The relevance of the mouse model for predicting interactions of I3C and TCDD in humans is unknown; however, the results suggest that human

health assessment of dietary PCDDs/PCDFs must take into account the contributions of other dietary AhR agonists or antagonists which act through common (AhR) pathways.

4. References

1. Bjeldanes, L. F., Kim, J. Y., Grose, K. R., Bartholomew, J. C., and Bradfield, C. A. (1991) Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol *in vitro* and *in vivo* - comparisons with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Proc. Natl. Acad. Sci. USA* 88:9543-9547.
2. Gillner, M., Bergman, J., Cambillau, C., Alexandersson, M., Fernström, B., and Gustafsson, J. A. (1993) Interactions of indolo[3,2-*b*]carbazoles and related polycyclic aromatic hydrocarbons with specific binding sites for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rat liver. *Mol. Pharmacol.* 44:336-345.
3. Jellinck, P. H., Forkert, P. G., Riddick, D. S., Okey, A. B., Michnovicz, J. J., and Bradlow, H. L. (1993) Ah receptor binding properties of indole carbinols and induction of hepatic estradiol hydroxylation. *Biochem. Pharmacol.* 43:1129-1136.
4. Vang, O., Jensen, m. B., and Autrup, H. (1990) Induction of cytochrome P450IA1 in rat colon and liver by indole-3-carbinol and 5,6-benzoflavone. *Carcinogenesis* 11:1259-1263.
5. Tiwari, R. K., Guo, L., Bradlow, H. L., Telang, N. T., and Osborne, M. P. (1994) Selective responsiveness of breast cancer cells to indole-3-carbinol, a chemopreventative agent. *J. Natl. Cancer Inst.* 86:126-131.
6. Liu, H., Wormke, M., Safe, S., and Bjeldanes, L. F. (1994) Indolo[3,2-b]carbazole: a dietary factor which exhibits both antiestrogenic and estrogenic activity. *J. Natl. Cancer Inst.* 86:1758-1765.
7. Stoewsand, G. S., Anderson, J. L., and Munson, L. (1988) Protective effect of dietary brussels sprouts against mammary carcinogenesis in Sprague-Dawley rats. *Cancer Lett.* 39:199-207.
8. Bradlow, H. L., Michnovicz, J. J., Telang, N. T., and Osborne, M. P. (1991) Effects of dietary indole-3-carbinol on estradiol metabolism and spontaneous mammary tumors in mice. *Carcinogenesis* 12:1571-1574.
9. Gierthy, J. F., Bennett, J. A., Bradley, L. M., and Cutler, D. S. (1993) Correlation of *in vitro* and *in vivo* growth suppression of MCF-7 human breast cancer by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Cancer Res.* 53:3149-3153.
10. Merchant, M., Morrison, V., Santostefano, M., and Safe, S. (1992) Mechanism of action of aryl hydrocarbon receptor antagonists: inhibition of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced *CYP1A1* gene expression. *Arch. Biochem. Biophys.* 298:389-394.
11. Jerne, N. K. and Nordin, A. A. (1963) Plaque-forming in agar by single antibody producing cells. *Science* 140:405
12. Cunningham, A. J. and Szenberg, A. (1968) Further improvements in the plaque technique for detecting single antibody-forming cells. *Immunology* 14:599-601.
13. Rittenberg, M. B. and Pratt, K. L. (1969) Antitrinitrophenyl (TNP) plaque assay. Primary response of Balb/c mice to soluble and particulate immunogen. *Proc. Soc. Exper. Biol. Med.* 132:575-581.
14. U.S.Environmental Protection Agency. (1994) Health Assessment for 2,3,7,8-TCDD and Related Compounds. External Review Draft. EPA/600/BP-92/001a-c.