Effects of Indole-3-carbinol on 3,3',4,4',5-Pentachlorobiphenyl-Induced Teratogenesis in Chicken Embryos and in C57BL/6 Mice

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1. Introduction

Polychlorinated biphenyls (PCBs) are widespread environmental chemicals. PCBs bioaccumulate in food chain, and the diet may be the major route of human exposure[1)2)]. The adverse human health effects of background levels of PCBs are variable and unknown; however, any assessment of possible adverse effects of these compounds should take into account dietary intakes of natural Ah receptor agonists, such as indole-3-carbinol (I3C), for their possible additive or non-additive effects. I3C, a compound that occurs naturally in large amounts in Brassica vegetables such as cabbage, broccoli, cauliflower and brussels spouts, represents another class of Ah receptor ligands distinct from the halogenated aromatic hydrocarbons (HAHs). Studies demonstrate that I3C and several acid condensation products bind to the Ah receptor and induce several Ah receptor-mediated responses including the induction of phase I and phase II enzyme activities in rodents[3)4)]. A recent study by Wilker et al. (1995)[5)] has shown that I3C altered male reproductive development similar to TCDD in Sprague-Dawley rats. I3C binds with low affinity to the Ah receptor and therefore it is possible that this weak Ah receptor agonist may also exhibit partial Ah receptor antagonist activity. The antagonist effects of I3C has been reported by Chen et al. (1995)[6)]. I3C inhibited TCDD-induced immunotoxicity and induction of CYP1A1 gene expression in B6C3F1 mice and Hepa 1c1c7 cells.

2. Objectives

The objective of this study was to evaluate the interactive effects of I3C on 3,3',4,4',5-pentachlorobiphenyl (3,3',4,4',5-pentaCB)-induced teratogenesis in the Chick Embryotoxicity Screening Test (CHEST) bioassay and in C57BL/6 mice.

3. Methods

Fertilized hen's eggs were incubated at 37.5°C and 80% relative humidity and turned every 6 hrs. On day 4 of incubation, test chemicals (dissolved in corn oil or corn oil plus 0.5% DMSO) were injected into the egg yolk in a volume according to the egg weight (maximum

volume less than 100 µl). Treatment groups included untreated control, solvent control, 3,3',4,4',5-pentaCB (ranging from 0.5 to 12.0 µg/kg egg weight), I3C (2.0 or 3.0 mg/kg), and 3,3',4,4',5-pentaCB (2.0 µg/kg) plus I3C (2.0 mg/kg). The eggs were candled every other day postinjection, and dead embryos were recorded and discarded. The experiment was terminated on day 18. Embryonic lethality was determined and surviving embryos were weighed and evaluated for malformations, pericardial and subcutaneous edema, and gross liver lesions. Liver tissues were fixed in 10% formalin solution for later histological examinations. The significance of the differences among the treatment groups was analyzed statistically by using a method for comparing two binomial populations. All statements of significance were at a probability level of $P \leq 0.05$ level.

Pregnant C57BL/6 mice were administered orally with a dose of 1044 µg 3,3',4,4',5-pentaCB/kg body weight either alone or in combination with 200 mg I3C/kg body weight on day 10 of gestation. Control animals were either treated with I3C (200 mg/kg), corn oil or left untreated. Body weights and general appearance of pregnant mice were monitored daily. Dams were euthanized on day 17 of gestation. The litter and liver weights were recorded. Number of implants, resorptions, dead and live fetuses were counted. Live individual fetuses were weighed and examined for gross malformations. The fetuses were fixed in Bouin's solution and examined for cleft palate and hydronephrosis. All data were subjected to analysis of variance using the General Linear Models Procedure of the Statistical Analysis System. The significance was determined by Waller-Duncan K-ratio *t*-test ($P \leq 0.05$ level).

4. Results

Exposure of chicken embryos to 3,3',4,4',5-pentaCB at concentrations ranging from 0.5-12.0 µg/kg egg weight resulted in dose-dependent increases in embryolethality, embryotoxicity and malformations (Table 1). At a concentration of 2.0 µg/kg, pentaCB resulted in 16% embryonic malformations, 56% edema and 63% liver lesions (Table 1). Histological examination of liver revealed subcapsular zones of coagulative necrosis with mineralization. I3C (2.0 mg/kg egg weight) did not induce any significant adverse effects in the embryos (Table 2). I3C (3.0 mg/kg) caused a significant increase in the embryo mortality. Cotreatment with 3,3',4,4',5-pentaCB (2.0 µg/kg) plus I3C (2.0 mg/kg) revealed neither agonist nor antagonist effects on pentaCB-induced teratogenic effects (Table 2).

There were no apparent signs of maternal and /or fetal toxicity in any of the groups treated with 3,3',4,4',5-pentaCB, I3C, or their combination in C57BL/6 mice. Significant differences in maternal liver/body weight ratios (i.e., an increase in the 3,3',4,4',5-pentaCB alone and in combination with I3C treatment groups, and a decrease in the I3C alone treatment group) were observed when compared to the control groups (data not shown). Treatment of mice with 3,3',4,4',5-pentaCB (1044 µg/kg) alone resulted in the induction of cleft palate (51%) and hydronephrosis (99%) (Table 3). I3C (200 mg/kg), did not produce any observable toxic effects in mice. Cotreatment of dams with 3,3',4,4',5-pentaCB (1044 µg/kg) + I3C (200 mg/kg) did not produce any significant effects (i.e., agonist or antagonist effects) on 3,3',4,4',5-pentaCB-induced teratogenesis.

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Table 1. Effects of 3,3',4,4',5-PentaCB on the Developmental Toxicity in Chicken Embryos.

Treatment	No. of eggs	Mortality (%)	Malformation (%)[1]	Edema (%)[1]	Liver lesions (%)[1]
Corn oil	104	12	3	0	0
PentaCB 0.5 µg/kg	19	5	0	0	11[a]
PentaCB 1.0 µg/kg	20	35[a]	15[a]	0	0
PentaCB 2.0 µg/kg	55	42[a]	16[a]	56[a]	63[a]
PentaCB 3.0 µg/kg	19	58[a]	62[a]	86[a]	75[a]
PentaCB 4.0 µg/kg	25	64[a]	67[a]	100[a]	100[a]
PentaCB 8.0 µg/kg	10	80[a]	100[a]	100[a]	100[a]
PentaCB 12.0 µg/kg	10	100[a]	N/A	N/A	N/A

[a] Values are significantly different from the solvent control (corn oil) ($p \leq 0.05$).
[1] The experiment was terminated on day 18 of incubation. The live fetuses were examined for anomalies and the percent values of malformation, edema and liver lesions were calculated on the total number of live embryos.

Table 2. Effects of 3,3',4,4',5-PentaCB, I3C and Their Combination in the Chicken Embryos.

Treatment	No. of eggs	Mortality (%)	Malformation (%)[1]	Edema (%)[1]	Liver lesions (%)[1]
Corn oil + 0.5% DMSO	20	20	0	0	0
PentaCB 2.0 µg/kg	20	70[a]	0	33[a]	50[a]
I3C 2 mg/kg	20	20	0	0	0
I3C 3 mg/kg	20	40[a]	0	0	8
PentaCB (2.0 µg/kg) + I3C (2 mg/kg)	20	55[a]	0	44[a]	44[a]

[a] Values are significantly different from the solvent control ($p \leq 0.05$).
[1] The experiment was terminated on day 18 of incubation. The live fetuses were examined for anomalies and the percent values of malformation, edema and liver lesions were calculated on the total number of live embryos.

Table 3. Teratogenic Effects of 3,3',4,4',5-PentaCB, I3C, and Their Combination in C57BL/6 Mice.

Treatment	No. of litters	No. of litters with cleft palate	Fetuses with cleft palate[1] (%)	Fetuses with hydrone-phrosis[1](%)
Control	7	0	0.00 ± 0.00[a]	0.0 ± 0.00[b]
Corn oil	10	0	0.00 ± 0.00[a]	0.0 ± 0.00[b]
I3C (200 mg/kg)	10	0	0.00 ± 0.00[a]	0.0 ± 0.00[b]
PentaCB (1044 µg/kg)	10	9	51.07 ± 38.04[b]	98.89 ± 3.51[a]
PentaCB (1044 µg/kg) + I3C (200 mg/kg)	10	9	66.66 ± 38.05[b]	100.00 ± 0.00[a]

[1] Values are means ± standard error.
Means with the same letter are not significantly different ($p > 0.05$).

Contrary to previous findings, the results from the present study demonstrated that I3C revealed neither agonist nor antagonist effects on 3,3',4,4',5-pentaCB-induced teratogenic effects both in the CHEST bioassay and in C57BL/6 mice. The agonist or antagonist effects of I3C may be species- and target organ-specific. The findings from the CHEST bioassay were in agreement with studies in C57BL/6 mice. The CHEST bioassay may be useful to delineate the interactions between complex mixtures of environmental toxins.

5. References

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