Opposite Effects of 2,2',4,4',5,5'-Hexachlorobiphenyl and 2,3,7,8-Tetrachlorodibenzo-p-dioxin on the Splenic Plaque-Forming Cell Response to Sheep Red Blood Cells in Female B6C3F1 Mice

Ralph J. Smialowicz, Michael J. Devito, Marie M. Riddle, Wanda C. Williams and Linda S. Birnbaum

U.S. Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, NC 27711, USA

Summary

The effect that co-treatment with 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has on the splenic primary antibody plaque-forming cell (PFC) response to sheep red blood cells (SRBC) was determined in female B6C3F1 mice. Groups of 8 mice/group were given a single oral dose of PCB153 alone (0, 3.58, 35.8 or 358 mg/kg), TCDD alone (0, 0.1, 1 or 10 μg/kg), and all possible combinations of these doses in corn oil 7 days prior to immunization with SRBC. Four days after immunization body, spleen, thymus and liver weights were measured and the PFC response to SRBC was determined. Exposure with TCDD alone resulted in a dose-related suppression of the PFC response, with significant suppression at 1 and 10 μg/kg. In contrast, exposure to PCB153 alone resulted in the enhancement of the PFC response at 358 mg/kg. Combined exposure to 358 mg/kg PCB153 and TCDD resulted in no change (PCB153 + 0.1 μg/kg TCDD) or suppression (PCB153 + 1 or 10 μg/kg TCDD) of the PFC response relative to PCB153 alone. However, the PFC response was enhanced (PCB153 + 0.1 μg/kg TCDD), unaffected (PCB153 + 1 μg/kg TCDD) or suppressed (PCB153 + 10 μg/kg TCDD) relative to corn oil controls. These results suggest that rather than acting as an antagonist of TCDD-induced immunosuppression, PCB153's enhancement of the PFC response alters the "set point" or "base line" response level such that co-treatment with TCDD at an immunosuppressive dose fails to suppress the PFC response relative to corn oil controls, while clearly suppressing it relative to the PCB153 control.

Introduction

Halogenated aromatic hydrocarbons (HAHs) are a group of industrial compounds or by-products which include the polychlorinated dioxins (PCDDs), biphenyls (PCBs) and dibenzofurans which occur as mixtures in the environment. A number of toxic and biochemical effects, which are believed to require initial binding to the aryl hydrocarbon (Ah) receptor, are produced in experimental animals following exposure to HAHs of which TCDD is the most potent. One of the most sensitive toxic endpoints of HAH exposure in mice is suppression of the splenic PFC response to SRBC following a single exposure to TCDD with an ED_{50} of approximately 0.7 μg/kg [1,2,3,4]. Certain structurally related HAHs which are approximate isostereomers of TCDD (e.g.,

coplanar and monoortho-coplanar PCB congeners), display Ah agonist activity and suppress the PFC response in mice [5,6]. On the other hand, certain complex PCB mixtures (i.e., Aroclors 1242, 1248, 1254 and 1260 [7,8]), PCBs (i.e., 2,2',4,4',5,5'-hexachlorobiphenyl [9] and 2,3,3',4,5'-penta- and 2,3,3',4,5,5'-hexachlorobiphenyl [6]), and a TCDF (i.e., 6-methyl-1,3,8-trichlorodibenzo-furan [10]) partially antagonize the immunosuppressive effects of TCDD in mice.

In this study, the potential for 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153) to antagonize TCDD-induced suppression of the splenic PFC response to SRBC[9] was reevaluated. Dose-response data for both TCDD and PCB153 effects on the PFC response, following single or simultaneous exposure of mice with TCDD and/or PCB153, were collected in order to better understand the interactions of these chemicals.

Methods

Chemicals: Dosing solutions of TCDD (>98% purity, Lot #MLB-15091-55, as determined by GC-MS, Radian Corp., Austin, TX) and PCB153 (>98% purity, Ultra Scientific, North Kingstown, RI) were prepared in corn oil.

Animals and Treatment: Female B6C3F1 mice (60-days-old) were obtained from Charles River Laboratory (Raleigh, NC) and allowed to acclimate 1 week prior to dosing. Mice were housed in shoe box-type polycarbonate cages containing heat-treated pine shavings (Beta Chips, North Eastern Products Inc., Warrensburg, NY) and given feed (Purina Lab Chow, Ralston Purina Co., St. louis, MO) and water ad libitum. An ambient temperature of 22°C, relative humidity of 55±5%, and a 12-hr light-dark cycle were provided. The animals were randomly assigned to treatment groups of 8 mice per group. TCDD alone (0, 0.1, 1, or 10 µg/kg), PCB153 alone (0, 3.58, 35.8, or 358 mg/kg), and all possible combinations were administered *per os*. At 7 days after dosing, all mice were immunized with an iv injection of 0.2 ml of 5% (i.e, 2 X 10^8) SRBC. Four days later, the mice were weighed and killed, and the thymus, spleen and liver were removed and weighed. Cell suspensions were prepared from the spleens and used in the primary antibody PFC response assay[1].

Statistics: Data were analyzed by one-way analysis of variance (ANOVA), with post hoc analysis using Dunnett's multiple comparison *t* test or the Student-Newman-Keuls test. A value of $p<0.05$ was considered significant.

Results and Discussion

Table 1 presents the data of the effects that TCDD alone (Expt. 1), PCB153 alone (Expt. 2), and TCDD + PCB153 at the highest dose (Expt. 3) have on relative thymus weight and the PFC response to SRBC. Thymus weights were reduced in mice dosed with TCDD alone at 1 and 10 µg/kg and at 10 µg/kg + 358 mgPCB153/kg relative to vehicle controls. In these same animals, the antibody responses to SRBC, expressed either as PFC/10^6 viable spleen cells or as PFC/spleen, were suppressed relative to vehicle controls. Serum anti-SRBC hemagglutination titers were also suppressed in these mice (data not shown). Mice dosed with 358 mgPCB153/kg had significantly enhanced PFC responses, with a 33-48% increase over vehicle controls. Co-administration of 1 µgTCDD/kg, which caused significant suppression of the PFC response when given alone (Expt. 1), and 358 mgPCB153/kg resulted in no suppression in the PFC response compared with the vehicle control (Expt. 3). However, when

compared to mice dosed with 358 mgPCB153/kg alone, which had an enhanced PFC response compared with vehicle controls, the PFC response was suppressed in mice exposed to 1 μgTCDD/kg + 358 mgPCB153/kg. Co-administration of 10 μgTCDD/kg and 358 mgPCB153/kg further suppressed the PFC response.

In conclusion, these results suggest that PCB153 is not an antagonist, in the strictest sense, of TCDD-induced immunosuppression in the mouse. The high dose of PCB153 enhanced the PFC response to the point where a normally immunosuppressive dose of TCDD (i.e., 1 μg/kg) did not appear to be effective when compared to corn oil control, although the PFC response was clearly suppressed when compared to PCB153 alone. Furthermore, PCB153 did not prevent suppression of this response, even when compared to corn oil controls, when co-administered with a high TCDD dose (i.e., 10 μg/kg).

[This abstract does not necessarily represent USEPA policy.]

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TABLE 1

The effect of exposure with TCDD or PCB153 alone or in combination on thymic involution and the splenic antibody plaque-forming cell response in female B6C3F1 mice

Expt. No.	TCDD (µg/kg)	PCB153 (mg/kg)	Mean Relative Thymus Weight ± SE	Mean PFC/10^6 spleen cells ± SE	Mean PFC ($X10^4$)/spleen ± SE
1	0	0	2.28 ± 0.07	1082 ± 146	18.55 ± 2.66
	0.1	0	2.11 ± 0.10 (93)[a]	966 ± 171 (89)	17.48 ± 3.58 (94)
	1	0	1.76 ± 0.14[b] (77)	668 ± 125[b] (62)	9.70 ± 1.51[b] (52)
	10	0	1.58 ± 0.10[b] (69)	362 ± 39[b] (33)	5.81 ± 0.62[b] (31)
2	0	0	2.70 ± 0.08	1173 ± 145	22.85 ± 3.31
	0	3.58	2.47 ± 0.15 (91)	1329 ± 213 (112)	25.47 ± 5.04 (110)
	0	35.8	2.35 ± 0.10 (87)	1021 ±66 (87)	18.47 ± 1.25 (81)
	0	358	2.72 ± 0.15 (102)	1740 ± 203[b] (133)	37.05 ± 4.62[b] (138)
3	0	0	2.20 ± 0.10	1411 ± 342	21.42 ± 3.62
	0	358	1.89 ± 0.17 (86)	2224 ± 172[d] (157)	41.33 ± 3.82[c] (148)
	0.1	358	2.28 ± 0.19 (104)	1836 ± 118 (123)	39.81 ± 4.22[c] (146)
	1	358	2.03 ± 0.21 (92)	1242 ± 146[d] (88)	23.44 ± 3.13[d] (109)
	10	358	1.58 ± 0.10[c] (72)	362 ± 39[c,d] (26)	5.81 ± 0.62[c,d] (27)

[a] Percent of 0 TCDD + 0 PCB control.
[b] $p<0.05$ vs 0 TCDD + 0 PCB control in experiments 1 and 2, Dunnett's multiple comparison *t*-test, n=8.
[c] $p<0.05$ vs 0 TCDD + 0 PCB control in experiment 3, Student-Newman-Keuls test, n=8.
[d] $p<0.05$ vs 0 TCDD + 358 mg/kg PCB control in experiment 3, Student-Newman-Keuls test.