Structure Dependent Effects of Polychlorinated Diphenyl Ether Congeners in C57BL/6 and DBA/2 Mice: Immunotoxicity and Monooxygenase Enzyme Induction.

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ABSTRACT

In vivo treatment of C57BL/6 (Ah responsive) mice with penta- or hexachlorinated diphenyl ether (pentaCDE, hexaCDE) congeners resulted in a dose dependent suppression of the splenic plaque forming cell (PFC) response and a corresponding increase of hepatic microsomal ethoxyresorufin-O-deethylase (EROD) activity. In vivo treatment of DBA/2 (Ah non-responsive) mice with the same congeners resulted in slight but significant humoral immune suppression and the induction of EROD activity but only at relatively high dose levels. These results are consistent with an Ah-receptor mediated response. In contrast, C57BL/6 mice treated with decachlorodiphenyl ether (decaCDE) showed that suppression of humoral immunity occurred at a dose of 10 umol/kg whereas doses of 100 and 400 umol/kg were required to significantly increase EROD activity. Preliminary results using DBA/2 mice showed that decaCDE caused minimal levels of immunosuppression and enzyme induction only at a dose of 400 umol/kg. These findings suggest that a component of humoral immune suppression caused by treatment with decaCDE is not dependent on the Ah receptor.

INTRODUCTION

Polychlorinated diphenyl ethers (PCDE) are contaminants of chlorophenol preparations which are widely used as wood preservatives, fungicides, slimicides, and bacteriostats. PCDE residues have been found throughout the environment in sediments, fish, wildlife and human tissues. PCDEs act similarly to other polychlorinated aromatic hydrocarbons in that they must bind to the aryl hydrocarbon (Ah) receptor to produce a response such as the induction of hepatic micosomal EROD activity.[1] This study investigates the structure-related immunotoxicity and enzyme activity of PCDE congeners in C57BL/6 (Ah responsive) and DBA/2 (Ah nonresponsive) male mice using the plaque forming cell response to sheep red blood cells or trinitrophenyl-lipopolysaccharide (TNP-LPS) and measurement of hepatic microsomal EROD activity.

MATERIALS AND METHODS

Chemicals PCDE congeners were synthesized in this laboratory as previously described.[2] Sheep red blood cells (SRBCs) in Alsevars solution were obtained from M.A. Bioproducts, Maryland, U.S.A. Guinea pig complement was purchased from GIBCO Laboratories, New York, U.S.A. All other chemicals used were the highest grade commercially available.
Animals Male C57BL/6 and DBA/2 mice were purchased at 7-8 weeks of age and were maintained on a 12 hour light/dark schedule with free access to food and water. The animals were allowed to acclimate 4 to 7 days before treatment. PCDE congeners were dissolved in corn oil and administered by i.p. injection. All animals were terminated by cervical dislocation.

Immunotoxicity Studies The "Cunningham" modification of the Jerne plaque forming cell (PFC) assay was used.[3,4] Five days after chemical treatment the animals (4-5 per group) were immunized with 4 x 10^8 SRBC and terminated 4-5 days later. A single cell suspension of spleen cells was prepared, washed and resuspended in RPMI 1640 media. For the preliminary studies DBA/2 mice were treated with decaCDE 4 days prior to immunization with 50ug TNP-LPS. SRBCs for the PFC assay were haptenated with TNP according to the method of Rittenberg and Pratt.[5] Viable spleen cells were counted by trypan blue staining.
EROD Enzyme Activity Hepatic microsomes were prepared and EROD activity was measured as previously described.[2]

RESULTS

Treatment of C57BL/6 mice with 2,3',4,4',5-pentaCDE, 2,2',4,5,5'-pentaCDE, 2,3,3',4,4'5-hexaCDE, or 2,2',4,4',5,5'-hexaCDE gave a dose dependent suppression of humoral immunity which correlated with an increase in EROD activity (Table 1). The most active congener for immunosuppression and enzyme induction was 2,3,3',4,4',5-hexaCDE followed by 2,3',4,4',5-pentaCDE, 2,2',4,4',5,5'-hexaCDE, and 2,2',4,5,5'-pentaCDE. The results in Table 1 also show that in DBA/2 mice the congeners also caused a dose dependent decrease in the splenic PFC response, however, significant effects were observed only at the highest doses (100 or 400 umol/kg) used in this study. Small but significant induction of EROD activity was observed at doses of 10 and 25 umol/kg for 2,3,3',4,4',5-hexaCDE and 2,3',4,4',5-pentaCDE, and at 400 umol/kg for 2,2',4,4',5,5'-hexaCDE. EROD activity was not induced by 2,2',4,5,5'-pentaCDE in DBA/2 mice.

Treatment of C57BL/6 mice with decaCDE resulted in immunosuppression which did not parallel enzyme induction (Table 1). DecaCDE caused a significant reduction in the splenic PFC response at a dose of 10 umol/kg whereas EROD induction was observed only at doses of 100 or 400 umol/kg. Preliminary studies with DBA/2 mice treated with 100 and 400 umol/kg doses of decaCDE and immunized with TNP-LPS were performed. DecaCDE significantly decreased the PFC response at both doses whereas only minimal but significant induction of EROD activity was observed. Body weights and splenic cellularity were not decreased by any of the treatments.

DISCUSSION

The structure-immunotoxicity and structure-induction relationships for several PCDE congeners in C57BL/6 mice (Table 1) are consistent with a role for the Ah receptor in mediating these responses. Moreover, in the relatively Ah non-responsive DBA/2 mice these responses are observed only at higher doses and these results are consistent with the effects observed for other structural classes of Ah receptor agonists. The results reported for decaCDE in C57BL/6 mice indicate that suppression of the splenic PFC response was a much more sensitive indicator of exposure than the induction of EROD activity. In DBA/2 mice both humoral immune suppression and induction were observed only at the highest doses (100 and 400 umol/kg). The differential responsiveness of C57BL/6 and DBA/2 mice are consistent with a role for the Ah receptor, however, based on structural considerations, decaCDE would not be expected to exibit Ah receptor agonist activity. Studies with other higher chlorinated PCDEs are currently in progress and will be used to further probe the role of the Ah receptor in mediating these effects.

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Table 1.

Dose Dependent Immunosuppressive and EROD Induction Activities of PentaCDE, HexaCDE, and DecaCDE in Ah Responsive C57BL/6 and Ah Non-responsive DBA/2 Mice

		C57BL/6		DBA/2	
Congener	Dose umol/kg	PFC/10^6 Viable Cells ± S.D.	EROD pmol/mg/min ± S.D.	PFC/10^6 Viable Cells ± S.D.	EROD pmol/mg/min ± S.D.
2,3,3',4,4',5-hexaCDE	0	2313±509	132 ± 22	862 ± 95	52 ± 6
	10	674 ± 158*	373 ± 77 *	702 ± 104	161 ± 21*
	25	356 ± 116*	957 ± 188*	637 ± 185	184 ± 88*
	100	195 ± 89 8	3060 ± 444*	271 ± 47*	850 ± 240*
2,3',4,4',5-pentaCDE	0	2437 ± 491	37 ± 10	808 ± 89	71 ± 12
	25	1034 ± 314*	1882 ± 149*	779 ± 134	116 ± 19*
	100	359 ± 60 *	2595 ± 315*	549 ± 142*	161 ± 22*
	400	202 ± 40 *	2985 ± 219*	297 ± 31*	326 ± 99*
2,2',4,4',5,5'-hexaCDE	0	2401 ± 416	191 ± 32	870 ± 57	106 ± 16
	25	1687 ± 381*	336 ± 37*	790 ± 230	93 ± 19
	100	857 ± 453*	823 ± 176	674 ± 106	120 ± 19
	400	265 ± 119*	2950 ± 590*	271 ± 103*	343 ± 113*
2,2',4,5,5'-pentaCDE	0	1356 ± 299	85 ± 21	947 ± 111	147 ± 26
	25	1184 ± 369	116 ± 15	941 ± 91	131 ± 24
	100	962 ± 193*	139 ± 26*	521 ± 155*	114 ± 24
	400	494 ± 289*	299 ± 44*	478 ± 114*	152 ± 20
decaCDE	0	1000 ± 96	181 ± 67	3703 ± 1016	107 ± 16
	2.5	956 ± 58	195 ± 31	nd[a]	nd
	10	443 ± 52**	258 ± 21	nd	nd
	25	376 ± 150**	319 ± 10	nd	nd
	100	157 ± 38**	632 ± 65**	1880 ± 580*	171 ± 32*
	400	63 ± 2**	2083 ± 442**	1783 ± 500*	303 ± 29*

* Significantly different ($p < 0.05$) from control values as determined by Student's t test.

**Significantly different ($p < 0.01$) from control values as determined by ANOVA.

[a] No Data