Immunosuppressive Activity of Alkyl-Substituted Polychlorinated Dibenzofurans in C57BL/6 Mice

L. Howie-Keller, R. Dickerson and S. Safe
Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX 77843-4466

1. Introduction

Alkylated polychlorinated dibenzofurans (PCDFs) are formed as by-products in pulp mill sludge and effluents [1]; however, these compounds are not routinely detected as environmental contaminants. Several studies have investigated the relative activities of 6-methyl-1,3,8-triCDF and related compounds as aryl hydrocarbon (Ah) receptor agonists and partial antagonists [2-5]. The results show that 6-methyl-1,3,8-triCDF is a weak Ah receptor agonist for most responses and, in animals or cells cotreated with 6-methyl-1,3,8-triCDF plus 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), there was significant inhibition of TCDD-induced CYP1A1/1A2 gene expression in rats, and porphyria, immunotoxicity and cleft palate in C57BL/6 mice [2-5]. In contrast, 6-methyl-1,3,8-triCDF did not antagonize TCDD-induced antiestrogenicity and exhibited Ah receptor agonist activity for this response in the rat uterus and in human breast cancer cells in culture [6-7]. Ongoing studies in this laboratory are investigating the antitumorigenic activity of alkyl PCDFs in rat and mouse mammary tumor models.

This report summarizes the structure-immunotoxicity and structure-induction relationships for a series of alkyl PCDFs containing 2, 3, or 4 lateral (2,3,7,8) substituents. The results show that congeners with the 2 lateral substituents exhibited the lowest activity for the two Ah receptor-mediated responses in C57BL/6 mice.

2. Materials and Methods

Chemicals. The alkyl PCDFs used in this study include: 6-methyl-1,3,8-triCDF and 8-methyl-1,3,6-triCDF (2 lateral substituents); 6-methyl-2,3,8-triCDF, 6-methyl-2,3,4,8-tetraCDF, 8-methyl-1,3,7-triCDF and 8-methyl-1,2,4,7-tetraCDF (3 lateral substituents); 7-methyl-2,3,8-triCDF and 8-methyl-2,3,4,7-tetraCDF (4 lateral substituents). The synthesis of these compounds will be described separately.

Immunotoxicity Assays. The alkyl PCDFs were dissolved in corn oil and administered (10 ml/kg) by intraperitoneal injection. Doses of the various congeners ranged from 0 to 400

μmol/kg; corn oil served as the vehicle control. Five days after treatment of C57BL/6 mice with the alkyl PCDFs (4 to 5 animals per treatment group), mice were challenged with sheep red blood cells (SRBCs) (4 x 10^8 cells per animal) [8,9]. Five days after antigen challenge, the animals were terminated by cervical dislocation, and the spleens were removed and placed immediately in ice-cold RPMI 1640 medium supplemented with 0.015 mM HEPES, 0.01 mM L-glutamine and antibiotics. A single cell suspension of each spleen was made by mashing between the frosted ends of two microscope slides. The spleen cells suspension was centrifuged at 220 *g* for 10 min and the cell pellets resuspended in 2 ml of fresh RPMI 1640 media. Aliquots of the spleen cell suspensions were then mixed with 0.20 ml of a 20% SRBC solution and 0.1 ml of a 20% guinea pig complement solution in phosphate-buffered saline. Approximately 35 ml of these mixtures were placed in a "Cunningham" chamber by capillary action and incubated for 1 hr at 37°C. The slides were then examined under low magnification and the number of lysis areas (plaques) was determined. The number of viable cells in each suspension was determined by trypan blue exclusion staining. The data was expressed as the number of plaque-forming cells (PFCs) per spleen and per 10^6 viable spleen cells.

Ethoxyresorufin O-deethylase (EROD) Activity. Hepatic microsomal EROD activity was determined fluorimetrically as previously described [10].

Statistical Analysis. A minimum of four animals was used for all treatment groups. Immunotoxicity data and enzyme activities were expressed as means ± SD. Significant differences between groups were determined either by the Student's t test or by ANOVA. ED_{50} values were determined by Logit transformation [11].

3. Results

The results presented in Table 1 indicate that the structure-immunotoxicity relationships for the alkyl PCDFs exhibited some similarities with the PCDFs. The least active compounds were the 6-methyl-1,3,8- and 8-methyl-1,3,6-triCDFs which were substituted in only 2 lateral positions. These compounds were also weak inducers of hepatic microsomal EROD activity and the relatively weak Ah receptor agonist activities for the 1,3,6,8-substituted compounds were comparable to results which have previously been reported. There were no consistent differences in the activities of alkyl PCDFs substituted with either 3 or 4 lateral substituents. For example, 8-methyl-2,3,4,7-tetraCDF was the most active congener and contained 4 lateral substituents; however, the second most active compound, 8-methyl-1,2,4,7-tetraCDF, is substituted in 3 lateral positions. The failure to observe larger structure-dependent differences in the immunotoxicity of the alkyl PCDFs may be due in part to the assay system. Previous studies have shown that inhibition of the PFC response to SRBCs, a T cell-dependent antigen, by halogenated hydrocarbons may also involve an Ah receptor-independent process [12]. Current studies are investigating the relative toxicities and antiestrogenic activities of alkyl PCDFs in rodents and developing these compounds as drugs for potential clinical use in treating mammary cancer. (Supported by the National Institutes of Health, CA64081).

Table 1. Immunotoxicity ED_{50} values and induction of EROD activity by alkyl PCDFs in C57BL/6 mice.

	ED_{50} Values (μmol/kg)		Maximal Induced EROD Activity[a] (dose, μmol/kg)
	PFCs/Spleen	PFCs/10^6 Viable Cells	
6-Methyl-1,3,8-triCDF	40.0	58.6	151 ± 37 (400)
8-Methyl-1,3,8-triCDF	17.1	24.3	226 ± 71 * (100)
6-Methyl-2,3,8-triCDF	14.3	9.2	671 ± 49* (25)
8-Methyl-1,3,7-triCDF	7.5	8.9	84 ± 8 (25)
8-Methyl-1,2,4,7-tetraCDF	3.9	5.3	101 ± 31 (25)
6-Methyl-2,3,4,8-tetraCDF	10.2	11.4	545 ± 112* (100)
8-Methyl-2,3,4,7-tetraCDF	2.9	3.6	228 ± 25* (10)
7-Methyl-2,3,8-triCDF	15.6	18.5	3299 ± 1566* (100)

[a] pmol/min/mg.
* Significantly induced at the highest dose used ($p < 0.05$).

5. References

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