ANTAGONISM BY DI-ORTHO POLYCHLORINATED BIPHENYLS OF Aryl HYDROCARBON RECEPTOR-DEPENDENT MODULATION OF CYP1A1 AND LPS-INDUCED IgM GENE EXPRESSION IN CH12.LX B CELLS

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Introduction

Halogenated aromatic hydrocarbons (HAHs) such as, polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs), are ubiquitous environmental pollutants [1]. A number of HAHs, of which TCDD is the most potent congener, produce a variety of adverse effects in laboratory animals. The adverse effects include hepatotoxicity, immune suppression, thymic atrophy, reproductive toxicity, wasting syndrome, endocrine disruption, developmental toxicity, and carcinogenicity [2]. Some of these toxic effects have been also observed in either wildlife or humans. HAHs in environmental and biological samples exist as complex mixtures of various congeners with different concentrations [3,4]. To estimate the potential toxicity of these complex mixtures, individual congeners have been assigned toxic equivalency factors (TEFs) which can be employed in a cumulative manner to yield toxic equivalents (TEQs) for HAH mixtures [1]. This approach assumes that the effects of individual HAH compounds in a mixture are essentially additive and their relative toxicity is proportional to their respective bind affinity to the Ah receptor [1,5]. Interestingly, some PCB congeners [PCB153 (2,2',4,4',5,5')] and commercial PCB mixtures (Aroclors) exhibit AhR antagonistic activities in several biochemical and toxic responses [6,7,8]. The non-additive interactions between different HAH compounds present in environmental samples suggest that the TEF approach may overestimate the effective TEQ for some responses. But the mechanism of this antagonism is unknown. In the present studies, we determined, in the CH12.LX murine B cell line [9], the effects of several di-ortho substituted PCB congeners on CYP1A1 and IgM gene expression modulated by TCDD and/or non-ortho coplanar PCB congeners. Furthermore, we have shown that antagonistic activity of di-ortho substituted PCBs occurs at the level of AhR activation and interference with AhR nuclear translocation.

Materials and Methods

Chemicals. All PCB congeners (>99.9% pure) and TCDD used in this study were purchased from AccuStandard (New Haven, CT) and dissolved in DMSO.

Cell Culture. The CH12.LX B cell line, derived from the murine CH12 B cell lymphoma, was cultured as described previously by Sulentic *et al.* [9].

Quantitative RT-PCR. Quantitative RT-PCR was performed as described previously by Williams *et al.* [10]. The number of transcripts were calculated from a standard curve generated from the

density ratio between the gene of interest and a specific internal standard concentration.

EMSA. Nuclear extracts were prepared as described by Williams *at al.* with several modifications [10]. The nuclear proteins (9 μ g) were incubated at 22°C for 15 min with 1 μ g of poly(dI-dC) in binding buffer [11]. Radiolabeled DRE oligomer was added (40,000 dpm) and incubated at 22°C for another 30 min .

Western blot analysis. Nuclear and cytosolic lysates prepared as described above from CH12.LX cells and subsequently resolved by denaturing SDS-PAGE with 7.5% polyacrylamide. Primary antibody to the AhR, as previously characterized by Pollenz *et al.* [11]. Detection was performed using the enhanced chemi-luminescence method.

ELISA. Supernatants were harvested from naive or LPS (3 μ g/ml)-stimulated CH12.LX cells after 72 hr incubation and were analyzed for IgM by sandwich ELISA as described by Sulentic *et al.* [9].

Results and Discussion

Relationship between the number of chlorination on the ortho position of PCB congeners and their effects on CYP1A1 mRNA expression

Initially, we measured the effect of several PCB congeners on TCDD-induced CYP1A1 mRNA expression using quantitative RT-PCR in CH12.LX cells. PCB77 (3,3',4,4'), which has no chlorinations in ortho positions, showed additive effect, whereas all di-ortho substituted congeners, including PCB153, showed inhibitory effect on TCDD-induced CYP1A1 expression. Among these, PCB52 (2,2',5,5') and PCB128 (2,2',3,3',4,4') showed stronger antagonistic activity of TCDD-induced CYP1A1 mRNA induction than PCB153. Conversely, mono-ortho substituted PCB congeners, PCB8 (2,4') and PCB105 (2,3,3',4,4'), did not produce marked changes in TCDD-induced CYP1A1 expression. Time-course studies showed that the antagonistic activity of di-ortho substituted congeners (PCB153 and PCB52) on CYP1A1 expression was detectable as early as 1 hr and most marked at 3 hr after co-treatment with TCDD. After 6 hr, the magnitude of antagonism of TCDD-induced CYP1A1 induction remained relatively constant (60-70% for PCB153 and more than 90% for PCB52) over the entire culture period. These results suggest that the antagonism by di-ortho substituted PCBs is persistent.

Antagonism of AhR ligand-induced inhibition of IgM expression by PCB52

The effects of di-ortho substituted PCB congeners on IgM secretion, which is dependent on AhR activation and represents one of the most sensitive indicators of toxicity produced by TCDD-like compounds, was evaluated by ELISA in LPS-activated cells [9,13]. In a preliminary experiment, PCB126 as well as TCDD did not suppress LPS-induced IgM secretion in the AhR deficient BCL-1 cell line [9]. In contrast, the AhR-expressing B cell line, CH12.LX exhibit significant inhibition of LPS-induced IgM secretion in the presence of TCDD. Interestingly, although PCB52, alone exhibited modest inhibition of IgM secretion, when co-treated with low concentration of TCDD (0.1, 0.3 nM), PCB52 antagonized TCDD-induced inhibition of IgM secretion. The antagonistic activity of PCB52 was most pronounced in the presence of 0.1 nM TCDD (both 25 and 50 μ M of PCB52 resulted significant antagonism) with a decrease in antagonism with increasing concentrations of TCDD. In contrast to CYP1A1 mRNA expression, antagonism of IgM secretion of by PCB52 was not observed in the presence of 1 nM concentration of TCDD. These results likely reflect the significantly greater affinity TCDD possess for AhR binding compared to PCB52 as suggested by the fact that 1 nM TCDD produces maximal

inhibition of LPS-induced IgM secretion in CH12.LX cells [9]. The antagonistic activity of PCB52 and the importance of agonist/antagonist ratio in IgM secretion of the response was more evident when increasing doses of PCB126 were used as an AhR agonist. PCB153 was also assessed for its antagonistic activity on IgM secretion, but it did not produce significant reversion. This is because high concentration (50μ M) of PCB153 itself showed weak inhibition on LPS-induced IgM secretion, and because antagonistic activity of PCB153 is relatively weak compared to that of PCB52. Additionally, compared to IgM secretion, CYP1A1 expression is a more sensitive indicator of AhR ligand-induced responses. While the maximal induction level of CYP1A1 is several hundred-fold, compared to vehicle control, the maximal inhibition of IgM secretion is less than 90%. 60 hr after LPS-activation, the mRNA expression level of IgM heavy chain in CH12.LX cells was evaluated using quantitative RT-PCR. Consistent with the IgM secretion response, PCB52 reduced TCDD- or PCB126-induced inhibition of IgM heavy chain mRNA expression.

Inhibition of TCDD-induced DRE binding and nuclear translocation of AhR by PCB52

DNA binding of AhR has been widely established to be responsible for many of TCDD-induced biochemical and toxic responses. To characterize further the mechanism of antagonism by di-ortho substituted PCBs of Ah receptor dependent gene expression, as exemplified by modulation of CYP1A1 and IgM, DNA binding activity of AhR/ARNT was assessed by gel shift assays. Nuclear proteins isolated from TCDD-treated CH12.LX cells exhibited an increase in DRE binding which increased with increasing concentrations of TCDD. PCB52 treatment of CH12.LX cells did not induce DRE DNA binding, but when CH12.LX cells were co-treated with TCDD, a marked decrease in TCDD-induced DRE binding was observed, which was concordant with TCDD-induced CYP1A1 mRNA expression. The inhibitory effect of PCB52 was most marked at 0.1 nM TCDD and diminished with increasing concentrations of TCDD. Since DRE binding by the AhR is preceded by AhR nuclear translocation, we assessed the effects of PCB52 on inhibition of TCDD-induced AhR nuclear translocation. Western blotting of nuclear protein from TCDD and PCB52 co-treated cells suggests that PCB52 inhibited TCDD-induced nuclear translocation of AhR. Interestingly, even in the absence of TCDD, PCB52 itself partially blocked the basal level of AhR nuclear translocation in CH12.LX cells.

The results presented in these studies demonstrate that di-ortho substituted PCB congeners inhibit AhR ligand-induced biological responses, such as modulation of CYP1A1 and LPS-induced IgM expression, in CH12.LX murine B cell line. Furthermore, these studies show the antagonism by di-ortho substituted PCBs is due to the decrease of transcriptionally active AhR complex in the nucleus of AhR ligand-treated cells. These findings suggest that di-othro substituted PCB antagonize the activation and nuclear translocation induced by high affinity AhR ligands. Moreover, because di-ortho substituted PCBs are commonly found as constituents in PCB mixtures present in the environment, it is possible that the current TEF/TEQ approach may overestimate the toxicity of complex HAH mixtures.

Acknowledgments

This work was supported in part by funds from NIEHS Grant ES02520.

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