THE EXPRESSION OF COX-2 AND PRO-INFLAMMATORY CYTOKINES INDUCED BY PCB IN HUMAN MAST CELLS REQUIRES NF-κB ACTIVATION

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Introduction

Polychlorinated biphenyls (PCBs), a xenobiotic group of halogenated aromatic hydrocarbons (HAHs) are widely spread environmental contaminants[1]. The recent evidence indicates that the immune system is a target for PCBs and is perhaps one of the most sensitive indicators for adverse PCB induced health effects[2,3]. Mast cells are involved in inflammatory reactions[4] by synthesizing and releasing a variety of mediator molecules including histamine, arachidonic acid metabolites and cytokines[5]. Cyclooxygenase (COX), which exists in two isoforms, COX-1 and COX-2, is the rate-limiting enzyme in the biosynthesis of prostagladins. COX-1 is constitutively expressed in nearly all cells while COX-2 is induced in a variety of cell types by diverse stimuli including cytokines, growth factors, and mitogens[6,7].

PCBs and other HAHs mediate their many toxic effects through the aromatic hydrocarbon receptor (AhR), a ligand activated transcription factor[8]. Upon agonist binding, the AhR heterodimerizes with the AhR nuclear translocator (ARNT) protein, which interacts with specific DNA sequences (5'-TNGCGTG-3'), termed xenobiotic responsive elements (XREs)[9] located upstream of responsive genes, resulting in their transcriptional expression[10,11]. Recent studies have shown that xenobiotic compounds such as 2,3,7,8-TCDD also may activate NF- κ B *in vivo* and in various cells in culture[12]. However, PCBs induced expression of COX-2 and endogenous cytokines in human mast cells has not been elucidated. We describe here the effect of PCB on the expression of COX-2 and endogenous cytokines by human mast cell line HMC-1 and its effect on transcription level in terms of NF-κB.

Materials and methods

1. Chemicals

2,2',4,4',5,5'-Hexachlorobiphenyl (PCB153), α-naphtoflavone, 8-methoxy psoralen, and pyrrolidine dithiocarbamate were purchased from Sigma Chemical Co (St. Louis, MO); AMV-reverse transcriptase, *Taq* DNA polymerase, oligo (dT)20 primer, deoxynucleotide triphosphate and RNase inhibitor by Takara Shuzo (Kyoto, Japan); poly [dI-dC], T4 polynuceotide kinase, and DNase I by Boehringer (Mannheim, FRG);[α-^{32}P]CTP and [γ-^{32}P]ATP by Amersham Buchler (Braunschweig, FRG). All other chemical reagents were from Merck (Hawthorne, N.Y.).

2. Cell culture and treatment

The human mast cell line HMC-1 (kindly provided by Dr J. H. Butterfield, Mayo Clinic, Rochester, MN) was cultured in IMDM (Isocove's modified Dulbecco's medium) (Sigma, St. Louis, MO). The cell line was passaged every 3-4 days and maintained under standard

conditions at 37℃, 5% CO_2.

3. Western blot

Cell lysates ($1x10^6$ cells) were subjected to SDS-PAGE using 10% gels. The proteins were electroblotted onto nitrocellulose membrane using a semidry blotter (BioRad, Hercules, CA). The membranes were sequentially incubated with COX-1 (goat) and COX-2 (rabbit) polyclonal antibodies (Cayman Chemical Co, Ann Arbor, MI) with 1/2000 dilutions for 2 h and horse radish peroxidase (HRP)-linked rabbit anti-goat IgG and goat anti-rabbit IgG (Zymed Laboratories, South San Francisco, CA) with 1/2000 dilutions for 1 h, then visualized using enhanced chemiluminescence system (Amersham, Arlington Heights, IL).

4. RNA isolation and RT-PCR

Total RNA was prepared from HMC-1 cell line ($2x10^6$ cells) using Trizol (Gibco-BRL, Gaithersburg, MD) according to manufacturer's instruction. First strand cDNA synthesis was performed using the Takara RNA PCR kit . PCR primers were purchased from R&D systems (human IL-4, IL-6) or BioSource International (Menlo Park, CA)(TNF α , GAPDH); all the others (IL-1β, COX-2, AhR) were designed and synthesized from Operon Technologies (San Diego, CA). Amplification of cDNA was performed by PCR using a thermal cycler (Perkin-Elmer, GeneAmp PCR system 2400; 30 cycles; 94℃for 1 min, 55-60℃ for 1 min and 72℃for 1 min). PCR products were revealed by ethidium bromide staining after separation on a 1-2% agarose gel.

5. Gel-shift assay analysis of NF-κB activation

Crude nuclear extracts were prepared from HMC-1 cell line ($1x10^6$ cells) and electrophoretic mobility shift assay (EMSA) was performed as described previously[13]. For analysis of NF-κB activation, the double-stranded oligonucleotide 5'-AGT TGA GGG GAC TTT CCC AGG C-3' (Promega Corp., Madison, WI) representing the consensus binding site and 5'-AGT TGA GGC GAC TTT CCC AGG C-3' representing the mutant was used. Specificity of shifted bands was examined by competition with the unlabeled oligonucleotide.

Results and Discussion

1. The expression of COX-2 and cytokine mRNAs

In Fig. 1B, PCB induced a maximal expression of COX-2 protein at 6 h and gradually decreased. However, PCB did not affect COX-1 protein expression. Since PCB-mediated induction of COX-2 protein could reflect the induction of transcription, we examined the COX-2 mRNA expression by RT-PCR (Fig. 1A). Treatment of the cells with PCB showed a maximal expression at 2 h and gradually decreased at 24 h. With the dose response condition, induction of COX-2 mRNA expression by PCB was shown dose-dependent manner (data known shown). Since various cytokines such as IL-1 β, IL-6, and TNF-α play important roles in the process of inflammation[14-16], we investigated IL-1β, IL-6, and TNF α mRNA expression in HMC-1 cells by PCB (Fig. 2). Unstimulated HMC-1 cells constitutively expressed TNF-α and IL-1 β. But when cells were treated with PCB, the expression of TNF-α mRNA was maximal increased at 24 h and IL-1 β mRNA expression was maximal at 12 h. The expression of IL-6 was induced by PCB to reach a peak at 2 h and was almost paralleled that of COX-2 mRNA in terms of time and duration of stimulant. Our studies clearly show the expression of PCB induced COX-2 and proinflammatory cytokines (TNF-α IL-1β, and IL-6) in HMC-1.

2. Effects of PCB treatment on NF-κB activation.

To investigate whether PCB-induced COX-2 and endogenous cytokines (TNF-α IL-6, IL-1β) are regulated by the DNA-binding activity of NF-κB transcription factor in HMC-1 cells, first we

have examined the effects of PDTC (an NF-κB inhibitor) on PCB-induced COX-2 and endogenous cytokine mRNA expression. As shown in Fig. 3, COX-2, IL-1β, TNF-α, and IL-6 mRNA expressions were absolutely decreased by PDTC, indicating PCB may induce target gene transcription mediated by NF- κ B. In order to confirm the NF-κB mediated gene transcription, we have investigated the effects of PCB-induced NF- κ B activity by EMSA in HMC-1 cells (Fig. 4). HMC-1 cells showed no endogenous NF-κB binding activity, but treated with PCB induced a dense shifted NF-κB binding band. The shifted band was competed with by the addition of wild type NF-κB consensus oligonucleotide (WT) but not by mutated NF-κB binding oligonucleotide (MT), indicating that the binding activity is specific to NF-κB. These results indicate that PCB can induce NF-κB activation in HMC-1 cells. The results in this study strongly suggest that PCB can induce inflammatory reaction through the induction of COX-2 and proinflammatory cytokines.

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Figure and Legend

Fig. 1. Time course expression of COX-2 mRNA and protein after stimulation of HMC-1 cells with PCB. Total RNA of cells treated for indicated times with 0.2% DMSO (Vehicle) or 60 nM PCB was prepared, reverse transcribed, and amplified by RT-PCR (A). Immunoblot analysis of COX-1 and COX-2 proteins in PCB-treated HMC-1 cell (B)

Fig. 2. Time course expression of transcripts for endogenous cytokines after stimulation of HMC-1 cells PCB. Total RNA of cells treated for indicated times with 0.2% DMSO (Vehicle) or 60 nM PCB was prepared, reverse transcribed and amplified by RT-PCR.

Fig. 3. Effects of PDTC, an NF-κB inhibitor on COX-2, IL-1β, IL-6, and TNF-α mRNA expression in PCB-treated HMC-1 cells. Cells were incubated for 30 min with 100 μM PDTC and 60 nM PCBs added. Total RNA of cells treated for indicated cases was prepared, reverse transcribed, and amplified by RT-PCR.

Fig. 4. Biding of an NF-κB consensus sequence to nuclear proteins in 60 nM PCB treated HMC-1 cells. DNA-binding activity of NF-κB was analyzed by EMSA using nuclear proteins extracted from PDTC or PDTC/PCB treated HMC-1 cells. MT (mutant type competitor), WT (wild type competitor)