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Ligand-independent activation of aryl hydrocarbon receptor signaling in PCB3-quinone treated HaCaT human keratinocytes

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HIGHLIGHTS

- 4-ClBQ, a metabolite of non-dioxin like PCB3 activates AhR signaling in HaCaT cells.
- 4-ClBQ-induced AhR activation is ligand-independent in HaCaT cells.
- 4-ClBQ-induced AhR activation is likely mediated by oxidative stress in HaCaT cells.

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ABSTRACT

Aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor that plays a critical role in metabolism, cell proliferation, development, carcinogenesis, and xenobiotic response. In general, dioxin-like polychlorinated biphenyls (PCBs) exhibit a ligand-dependent activation of AhR-signaling. Results from this study show that a quinone-derivative (1-(4-Chlorophenyl)-benzo-2,5-quinone; 4-ClBQ) of a non-dioxin like PCB (PCB3) also activates AhR-signaling. Treatments of HaCaT human keratinocytes with 4-ClBQ and dioxin-like PCB126 significantly increased AhR-target gene expression, CYP1A1 mRNA and protein levels. 4-ClBQ-induced increase *CYP1A1* expression was associated with an increase in the nuclear translocation of AhR protein as well as an increase in the luciferase-reporter activity of a human *CYP1A1* xenobiotic response element (XRE). 6,2′,4′-Trimethoxyflavone (TMF), a well-characterized AhR-ligand antagonist significantly suppressed PCB126-induced increase in *CYP1A1* expression, while the same treatment did not suppress 4-ClBQ-induced increase in *CYP1A1* expression. However, siRNA-mediated down-regulation of AhR significantly inhibited 4-ClBQ-induced increase in *CYP1A1* expression, suggesting that AhR mediates 4-ClBQ-induced increase in *CYP1A1* expression. Interestingly, treatment with the antioxidant *N*-acetyl-L-cysteine significantly suppressed 4-ClBQ-induced increase in *CYP1A1* expression. Furthermore, *CYP1A1* expression also increased in cells treated with hydrogen peroxide. These results demonstrate that a ligand-independent and oxidative stress dependent pathway activates AhR-signaling in 4-ClBQ treated HaCaT cells. Because AhR signaling is believed to mediate xenobiotics response, our results may provide a mechanistic rationale for the use of antioxidants as effective countermeasure to environmental pollutant-induced adverse health effects.

1. Introduction

Aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor and a member of the basic helix-loop-helix (bHLH)/Per-ARNT-Sim (PAS) superfamily of transcription factors (Burbach et al., 1992; Poland et al., 1976). Exogenous ligands of AhR include dioxin-like polychlorinated biphenyls (PCBs), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and halogenated aromatic hydrocarbons (Puga et al., 2009). AhR in the cytosol is inactive due to its binding to the chaperone proteins (HSP90, p23, and XAP2).

Abbreviations: 4-ClBQ, 1-(4-Chlorophenyl)-benzo-2,5-quinone; AhR, aryl hydrocarbon receptor; ARNT, aryl hydrocarbon receptor nuclear translocator; CYP1A1, cytochrome P4501A1; NAC, *N*-acetyl-L-cysteine; PCB3, 4-monochlorobiphenyl; PCB77, 3,3′,4,4′-tetrachlorobiphenyl; PCB126, 3,3′,4,4′,5-pentachlorobiphenyl; PCB169, 3,3′,4,4′,5,5′-hexachlorobiphenyl; PCB153, 2,2′,4,4′,5,5′-hexachlorobiphenyl; PCBs, polychlorinated biphenyls; ROS, reactiveoxygen species; XRE, xenobiotic response element; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; TMF, 6,2′,4′-trimethoxyflavone.

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Following binding to ligand, AhR undergoes a conformational change resulting in its translocation to the nucleus. In the nucleus, AhR dissociates from the chaperone proteins and binds to another bHLH/PAS family protein, aryl hydrocarbon receptor nuclear translocator (ARNT) (Hoffman et al., 1991; Puga et al., 2009). The AhR–ARNT heterodimer then binds to the xenobiotic response element (XRE, core DNA sequence: 5′-GCGTG-3′), which results in the enhancement of gene transcription (Denison et al., 1988; Puga et al., 2009). An increase in the expression of cytochrome P4501A1 (CYP1A1) that has XRE sequence in its promoter enhancer region is routinely used as an indicator of the activation of the AhR-signaling pathway. In addition to the exogenous ligands, endogenous ligands of AhR (*e.g.,* lipoxin A4, bilirubin, and biliverdin) have also been identified (Phelan et al., 1998; Schaldach et al., 1999). Furthermore, tryptophan and its metabolites are also believed to be the endogenous ligands activating AhR-signaling and enhancing the expression of *CYP1A1* in UV-irradiated HaCaT cells (Fritsche et al., 2007). Whereas the majority of the studies report ligand-dependent activation of the AhR-signaling pathways, Chang et al., (2007) have shown a ligand-independent activation of the AhR-signaling pathway. These authors have found that the cellular proliferation was faster in AhR-null mouse embryonic fibroblasts (MEFs) expressing AhR-wt and a ligand-binding domain deleted-AhR gene compared to the proliferation of AhR-null MEFs. These previous reports suggest that both ligand-dependent and ligand-independent pathways can activate AhR-signaling.

Polychlorinated biphenyls (PCBs) are a group of persistent organic pollutants that are ubiquitously found in the environment and they are known to have adverse health effects (Lauby-Secretan et al., 2013). PCBs have 209 possible congeners, among which 12 are dioxin-like PCBs and the remaining 197 are non-dioxin like PCBs (Henry and DeVito, 2003). Dioxin-like PCBs have high affinity for the AhR compared to non-dioxin like PCBs that are believed to have no affinity or very low affinity for binding to AhR (Henry and DeVito, 2003). Exposure to dioxin-like PCBs (3,3′,4,4′-tetrachlorobiphenyl, PCB77; 3,3′,4,4,5,5′-hexachlorobiphenyl, PCB169) increases the enzymatic activity of cyp1a1 that was associated with an inhibition in intracellular communication in mouse Hepa1c1c7 cells. Such an inhibition in intracellular communication was absent in PCB77 and PCB169 treated AhR-mutant cells (De Haan et al., 1994). 3,3′,4,4′,5-Pentachlorobiphenyl (PCB126) is the most potent AhR agonist among PCBs (Kafafi et al., 1993). Treatment with PCB126 significantly stimulates the expression of hepatic *CYP1A1* in Sprague-Dawley rats, while treatments with the non-dioxin like PCB 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB153) had no effect on *CYP1A1* expression (Vezina et al., 2004). These previous reports suggest that dioxin-like PCBs are ligands for AhR resulting in the activation of the expression of the AhR-target gene, *CYP1A1*.

Results from this study show that an AhR ligand-independent and oxidative stress-dependent pathway activates AhR-signaling in HaCaT cells treated with PCB3-quinone, a metabolite of non-dioxin like PCB, PCB3.

2. Materials and methods

2.1. Chemicals, reagents, and antibodies

PCBs (4-ClBQ and PCB126, Fig. 1) were provided by the Synthesis Core of the Iowa Superfund Research Project. These compounds were synthesized and purified as described previously (Lehmler and Robertson, 2001). Stealth negative control and human AhR siRNAs were obtained from Invitrogen (Life Technologies, Grand Island, NY). 6,2′,4′-Trimethoxyflavone (TMF) and *N*-acetyl-L-cysteine (NAC) were purchased from Sigma Chemical Co. (St. Louis, MO). Antibodies against human AhR (ab153744) and CYP1A1 (ab3568) were obtained from Abcam (Cambridge, MA); β-actin antibody (sc47778) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA); Lamin A/C antibody (cst4777S; Cell Signaling Technology) was a generous gift from Dr. Jennifer L. Casey, Department of Internal Medicine, University of Iowa.

2.2. Cell cultures and treatments

Spontaneously immortalized human skin keratinocytes (HaCaT) provided by Dr. Norbert Fusenig (German Cancer Research Center, Heidelberg, Germany) (Boukamp et al., 1988) were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum at 37 °C in 5% CO_2. Dimethyl sulfoxide (DMSO) was used to prepare stock solutions of PCBs (Venkatesha et al., 2008). Monolayer cultures were treated with 0–3.0 μM of PCBs for 24 h in serum-free DMEM. Control cells were treated with the equivalent amount of DMSO (0.1%, v/v) in absence of PCBs.

2.3. cDNA synthesis and quantitative RT-PCR assay

Total RNA of control and PCB treated cells was extracted using TRIzol (Invitrogen, Carlsbad, CA). ND1000 Nanodrop spectrophotometer (Nanodrop, Wilmington, DE) was used to measure the concentration and purity of RNA. One microgram of RNA was reverse transcribed using cDNA Archive Kit (Applied Biosystems, Carlsbad, CA). Eighty nanogram of the cDNA were used to perform real-time PCR amplification using Power SYBR Green PCR Master Mix and StepOnePlus™ System (Applied Biosystems, Carlsbad,

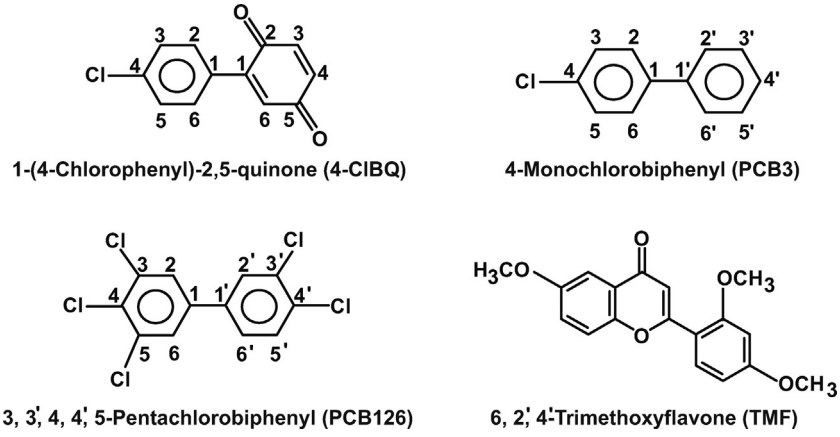

Fig. 1. Structure of chemicals used in this study.