PCB126 disrupts epidermal growth factor internalization and receptor phosphorylation

Hardesty JE[1], Hongxue S[2], Jin J[2], Clair HB[1], Falkner KC[3], Prough RA[1], Cave MC[1,2,3].

[1] Department of Biochemistry and Molecular Genetics, University of Louisville, Louisville, KY, United States, 40245
[2] Department of Pharmacology and Toxicology, University of Louisville, Louisville, KY, United States, 40245
[3] Division of Gastroenterology and Nutrition, University of Louisville, Louisville, KY, United States, 40245

Introduction

Polychlorinated biphenyls (PCBs) are characterized as endocrine and metabolism disrupting chemicals (EDCs/MDCs)(1). While they have been banned from production in the US since 1979 they still persist in humans through consumption of contaminated biota(2). PCB exposures have been associated with endocrine and metabolic disease including diabetes, obesity, and fatty liver disease(3). PCBs also impact developing and wound healing(4-8). The molecular aberrations due to PCBs and their contribution to these disease states are ill-defined. PCBs have been characterized as dioxin-like (DL) or nondioxin-like (NDL) based on their ability to activate the aryl hydrocarbon receptor (AhR). PCB126 is a well characterized AhR agonist but has been shown to upregulate constitutive androstane receptor (CAR) target genes as well(9). PCB126 may interact with other receptors including the constitutive androstane receptor (CAR) or epidermal growth factor receptor (EGFR)(10). Recently the CAR activation mechanism by xenobiotics has become more complex as there is an accepted direct and indirect CAR activation pathway(10,11). The direct activation pathway is through direct ligand activation by canonical CAR ligands TCPOBOP (mice) and CITCO (humans). The indirect pathway is through inhibition of the epidermal growth factor receptor (EGFR) leading to loss of CAR phosphorylation and thus, its activation(11). This would help explain the metabolic diseases associated with PCB exposure as EGFR inhibition can promote inflammation, worsened steatosis, diminish insulin secretion, and wound healing capabilities(12-19). This is a currently under developed area of research which may explain how PCB126 can promote CAR activation(9). This expands the mechanism for adverse effects due to PCB126 exposure since many adverse effects due to EGFR inhibition coincide with PCB126 exposure. These adverse effects include skin inflammation, liver injury, pancreatic dysfunction, and developmental disorders(3-9,14,20-31).

Materials and Methods

PCB-126 was purchased from Accustandard (New Haven, CT). Fluorescent-labeled epidermal growth factor was purchased from Thermo Scientific (Philadelphia, PA). A-431 cells (human epidermoid carcinoma) were acquired from American Type Culture Collection (ATCC) and cultured under the supplier's recommendation. A-431 cells were pre-treated with PCB-126 at varied logarithmic concentrations followed by treatment with fluorescently labeled EGF. The reaction was conducted at 37° C for 30 minutes. Cells were then washed, fixed, and nuclei stained. Fluorescent images were recorded and quantified with the ArrayScan instrument (Thermo Scientific). The quantitative measure was fluorescent EGF per cell. EGFR phosphorylation and total EGFR were measured by western blot analysis and antibodies were purchased from Cell Signaling Technology (Danvers, MA).

Results and Discussion

PCB126 appears to act at two sites in ablation of EGF endocytosis. It is potent at disrupting low affinity state binding of EGF to the EGFR monomer (femtomolar range) (Fig 1). While less potent at disrupting high affinity binding of EGF to EGFR pre-formed dimers, it is still relatively potent (nanomolar). The EGF assay was validated

by measuring EGFR phosphorylation by western blot analysis as well (Fig 2). This is a novel mechanism of action for PCB126 and could have implications on adverse effects due to PCB126 exposure. Our data suggest that PCB126 mediated loss of EGF internalization and phosphorylation is far more potent than what has been characterized for activation of AhR. This disruption of EGF signaling by PCB126 is at environmentally relevant concentrations. Further investigation of AhR and EGFR crosstalk should be pursued to identify the regulatory mechanisms[31]. More research is necessary to determine if the biphasic response is due to inhibition of monomeric vs multimeric EGFR or rather two distinct binding sites within the monomeric EGFR.

Figure 1

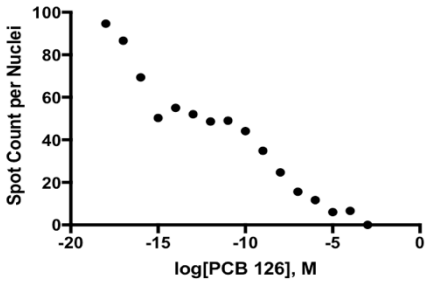

Fig 1. PCB126 is a potent disruptor of epidermal growth factor internalization. A-431 cells were incubated with PCB126 at various concentrations then followed by incubation with fluorescent labeled EGF. EGF spots per Nuclei were measured by fluorescence microscopy with the ArrayScan instrument. Each value is the mean value of a n=8.

Figure 2

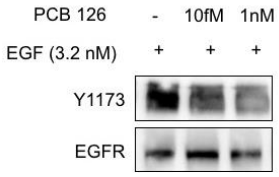

Fig 2. PCB126 prevents EGFR phosphorylation. A-431 cells were incubated with PCB126 at either 10 fM, 10 nM or no PCB126 followed by incubation with 3.2 nM of EGF. EGFR phosphorylation at Y1173 was measured by western blot analysis.

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