Toxicology and Applied Pharmacology 339 (2018) 24–33

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/taap

Intestinal exposure to PCB 153 induces inflammation *via* the ATM/NEMO pathway

Matthew C. Phillips[a], Rishu Dheer[b], Rebeca Santaolalla[b], Julie M. Davies[b], Juan Burgueño[b], Jessica K. Lang[b], Michal Toborek[c], Maria T. Abreu[a,b,*]

[a] Department of Microbiology and Immunology, University of Miami Miller School of Medicine, Miami, FL, USA
[b] Division of Gastroenterology, Department of Medicine, University of Miami Miller School of Medicine, Miami, FL, USA
[c] Department of Biochemistry and Molecular Biology, University of Miami Miller School of Medicine, Miami, FL, USA

ARTICLE INFO

Keywords:
Polychlorinated biphenyl
PCB 153
Intestinal inflammation
NF-κB
ATM
Nemo

ABSTRACT

Background: Polychlorinated biphenyls (PCBs) are persistent organic pollutants that adversely affect human health. PCBs bio-accumulate in organisms important for human consumption. PCBs accumulation in the body leads to activation of the transcription factor NF-κB, a major driver of inflammation. Despite dietary exposure being one of the main routes of exposure to PCBs, the gut has been widely ignored when studying the effects of PCBs.

Objectives: We investigated the effects of PCB 153 on the intestine and addressed whether PCB 153 affected intestinal permeability or inflammation and the mechanism by which this occurred.

Methods: Mice were orally exposed to PCB 153 and gut permeability was assessed. Intestinal epithelial cells (IECs) were collected and evaluated for evidence of genotoxicity and inflammation. A human IEC line (SW480) was used to examine the direct effects of PCB 153 on epithelial function. NF-κB activation was measured using a reporter assay, DNA damage was assessed, and cytokine expression was ascertained with real-time PCR.

Results: Mice orally exposed to PCB 153 had an increase in intestinal permeability and inflammatory cytokine expression in their IECs; inhibition of NF-κB ameliorated both these effects. This inflammation was associated with genotoxic damage and NF-κB activation. Exposure of SW480 cells to PCB 153 led to similar effects as seen *in vivo*. We found that activation of the ATM/NEMO pathway by genotoxic stress was upstream of NF-kB activation.

Conclusions: These results demonstrate that oral exposure to PCB 153 is genotoxic to IECs and induces downstream inflammation and barrier dysfunction in the intestinal epithelium.

1. Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous and persistent organic pollutants that adversely affect human health (Quinete et al., 2014). Although industrial production of PCBs has been discontinued, they remain a pressing environmental problem due to their slow biodegradation and high lipophilicity. These properties enable PCBs to bioaccumulate in food chains leading to high levels of PCBs in the tissues of economically important foodstuffs, especially fish. PCB 153, a non-coplanar PCB, has been shown to be especially prevalent in the environment and in fish consumed by humans (Fitzgerald et al., 2007). Indeed, the people at the highest risk of adverse PCB related effects are those from high fish-consuming populations (Barone et al., 2014). PCB 153 constitutes the bulk of PCB congeners found in humans—making it a vital PCB to study (Kraft et al., 2017). Once in the body, PCBs accumulate in various organs leading to inflammation (Sipka et al., 2008). These populations are at risk for a variety of health concerns associated with inflammation; such as cancer, metabolic syndrome, and endocrine dysfunction (Arrebola et al., 2014; Kashima et al., 2015). Despite these risks, and the fact that dietary exposure is one of the main routes of exposure to PCBs, the human gastrointestinal tract has been widely ignored when studying the effects of PCBs. One notable exception to this oversight is the reported increase in intestinal permeability after oral PCB exposure in mice (Choi et al., 2010).

Previous work by our group has shown that when acutely exposed to PCBs, including PCB 153, intestinal barrier function is compromised allowing for lipopolysaccharide (LPS) to leak into the systemic circulation (Choi et al., 2012). *In vitro* experiments have also shown that when exposed to PCBs, intestinal epithelial cell (IEC) line monolayers increase their permeability and alter regulation of junctional proteins (Choi et al., 2010). Disruption of the intestinal barrier is seen in many inflammatory diseases such as inflammatory bowel disease, metabolic

* Corresponding author at: Division of Gastroenterology, 1011 NW 15th Street, Suite 511, Miami, FL 33136, USA.
E-mail address: Mabreu1@med.miami.edu (M.T. Abreu).

https://doi.org/10.1016/j.taap.2017.11.027
Received 5 September 2017; Received in revised form 8 November 2017; Accepted 28 November 2017
Available online 29 November 2017
0041-008X/

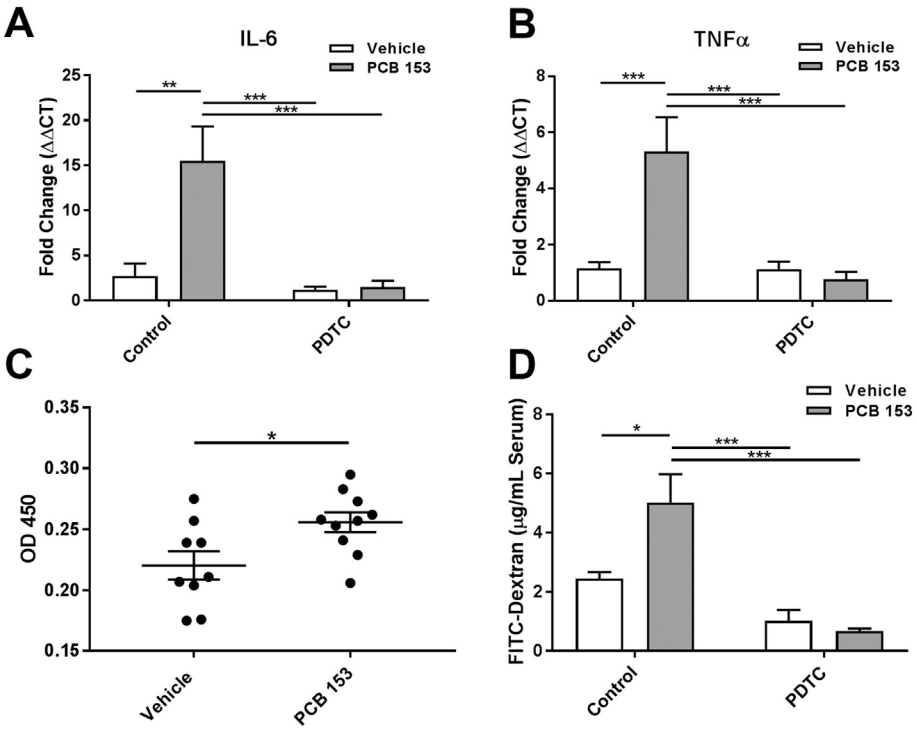

Fig. 1. PCB 153 causes NF-κB mediated intestinal inflammation and increases gut permeability. Mice were orally exposed once per day to PCB 153 (300 μmol/kg) or vehicle for 2 days, following IP injections of the NF-κB inhibitor PDTC or control (PBS).
(A.) IECs were isolated, RNA was extracted and RT-qPCR run for IL-6 and (B.) TNFα. N = 5 mice per group. Two-way AVOVA with Tukey's multiple comparison test. **P < 0.01, ***P < 0.001.
(C.) IECs were isolated and the nuclear fraction was extracted. A NF-kB binding assay was performed. (N = 9–10) Unpaired, two-tailed *t*-test. *P < 0.05.
(D.) 4 h prior to sacrifice, mice were orally gavaged with 60 mg/100 g body weight of FITC-dextran. At the time of euthanasia, serum was collected and levels of FITC-dextran were quantified. N = 5 mice per group. Two-way AVOVA with Tukey's multiple comparison test. *P < 0.05, ***P < 0.001.

syndrome, celiac disease and multiple sclerosis (Arrieta et al., 2006). This association between inflammation and intestinal barrier dysfunction, along with reports of PCB-induced inflammation in other tissues led us to suspect PCBs may have pro-inflammatory effects on the intestinal epithelium.

IECs are rapidly dividing, line the gastrointestinal tract, and are some of the first cells exposed to toxins released from foods. Research on other rapidly dividing cell lines, such as gonadal fibroblast from trout, have shown that PCB 153 causes damage to DNA (Marabini et al., 2011). In other settings, genotoxic damage can activate the transcription factor NF-κB, a major driver of inflammation, through ataxia telangiectasia mutated (ATM), a kinase activated by DNA damage, and the NF-κB essential modulator (NEMO) (Wu et al. 2006). PCB 153 has been shown to increase NF-κB nuclear localization and DNA binding in the liver of mice following intraperitoneal exposure (Lu et al., 2004). Additionally, PCB 153 has been shown to upregulate inflammatory genes, such as interleukin (IL) -6, and tumor necrosis factor-α (TNF-α), in an NF-κB dependent manner in mast cells (Kwon et al., 2002). Taken together, this evidence led us to hypothesize that PCB 153 causes DNA damage, leading to the activation of NF-κB, which drives inflammation and barrier dysfunction in the intestine.

The aim of the current study was to characterize the response of IECs to PCB 153. Although it is known that PCB exposure occurs through the intestine, there is a dearth of information on the effect of acute oral exposure of PCB153 on the intestinal epithelium. Specifically, we wanted to explore if PCB 153 causes inflammation in IECs. We then wanted to elucidate if genotoxic activation of NF-κB could be one mechanism for the inflammation and barrier dysfunction associated with PCB 153 exposure. In a series of *in vitro* and *in vivo* studies, we show that PCB 153 causes genotoxic damage to IECs, leading to the activation of NF-κB, upregulation of inflammatory cytokines, and an increase in intestinal permeability.

1.1. Materials and methods

1.1.1. Chemicals and reagents

PCB 153 (2,2′,4,4′,5,5′-hexachlorobiphenyl) was purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in dimethyl sulfoxide (DMSO, EMD Millipore Corp., Billerica, MA) to make the stock solution (10 mM). For *in vivo* use, PCB 153 stock solution was diluted in safflower oil. The same amount of DMSO diluted in safflower oil was used a negative control. Etoposide was purchased from Sigma-Aldrich (St. Louis, MO) and LPS was purchased from Invivogen (San Diego, CA). Etoposide, a topoisomerase II inhibitor, was used as a positive control for both genotoxic damage and for genotoxic activation of NF-κB *via* ATM and NEMO, as etoposide was the agent first used to elucidate this mechanism (Wu et al. 2006). LPS was used as a positive control for NF-κB activation by the canonical, non-genotoxic pathway. The free radical scavenger, N-acetylcysteine (NAC), the NF-κB inhibitor, pyrrolidine dithiocarbamate (PDTC), and the inhibitor of genotoxic NF-κB activation, *Clostridium difficile* Toxin B, were all purchased from Sigma-Aldrich (St. Louis, MO). The ATM inhibitor, KU-55933 was purchased from Abcam (Cambridge, United Kingdom).

1.1.2. Cell culture and PCB 153 treatment

The human intestinal cell line SW480 (ATCC CCL-228) was used for all *in vitro* experiments. SW480s were chosen because of their high level of responsiveness to other activators of NF-κB such as LPS. Cells were grown at 37 °C in Dulbecco's Modified Eagle Medium (DMEM, Corning, Corning, NY) supplemented with 10% fetal bovine serum (Gemini Bio Products, West Sacramento, CA) and 1% Penicillin-Streptomycin (Sigma-Aldrich, St. Louis, MO). For experiments, DMEM without FBS was used. For inhibition experiments, cells were pretreated for 1 h with either 5 mM NAC or 10 μM of KU-55833; or for 2 h with 100 ng/mL or 50 ng/mL of *C. difficile* Toxin B, before PCB 153 exposure. Cells were exposed to either 50 ng/mL LPS, 10 μM or 1 μM Etoposide, 100 μM, 50 μM, or 10 μM PCB 153, or DMSO. Dosages of PCB 153 were chosen based on the range of possible concentrations that intestinal epithelial cells would be exposed to after a meal high in PCBs (Desvignes et al., 2015). Cells were exposed for 3 h and then collected for analysis. Treatments at these dosages did not affect cell growth or viability of the cells as determined by an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (ATCC, Manassas, VA) performed as per manufacturer's instructions (supplemental Fig. 1).