CHARACTERIZING THE PPAR γ LIGAND BINDING POTENTIAL OF SEVERAL MAJOR FLAME RETARDANTS AND THEIR METABOLITES

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Introduction

Obesity has become a world-wide epidemic over the past few decades. According to a report from the Center for Disease Control and Prevention (CDC), 17% of children between 2 and 19 years old are obese in the US[1]. While genetic inheritance, diet, and exercise certainly contribute to obesity, recent studies have showed that prenatal exposures to "environmental obesogens" including phthalates, organotins and perflourinated compounds may increase odds of obesity in children[2]. Current research also suggests that many of these chemicals compounds act via a mechanism that includes activation of peroxisome proliferator-activated nuclear receptors (PPARs)[3]. PPARs are master transcriptional regulators, controlling the regulation of genes and pathways that control intracellular lipid flux, and adipocyte proliferation and differentiation. Together with the retinoid X receptor (RXR), they serve as metabolic ligand sensors for a variety of hormones, dietary fatty acids, and their metabolites[4]. Agonism of PPAR γ in particular is associated with lipid biosynthesis and storage, initiating differentiation of pre-adipocytes to adipocytes. Therefore, chemicals that specifically activate (agonize) PPAR γ and upregulate expression, may be more likely to impact development of obesity, if chronic environmental exposures are playing a role. In a recent study, pre-and postnatal exposure to the flame retardant mixture Firemaster 550 (FM550), a major replacement for pentabromodiphenyl ether (Penta-BDE), resulted in a 20-30% weight gain in male and female rats relative to controls[5]. Here, we build upon this study by exploring the potential ligand binding of FM 550 components (and their metabolites) with PPAR γ. We also investigate the potential binding of other brominated and organophosphate flame retardants that are ubiquitous contaminants in indoor environments. Furthermore, PPAR γ binding activities of environmental relevant dust samples which contain those chemicals and some other organic contaminants were also examined in this study.

Materials and methods

In this study, a commercially available high throughput assay, (PolarScreen™ PPARγ-competitor assay kit, Invitrogen) was used to investigate the binding potency of several important flame retardants (FRs) and their metabolites to the PPARγ protein. The kit uses the human-derived recombinant PPARγ ligand binding domain (PPARγ-LBD) tagged with a N-terminal GST-tag, and a selective fluorescent PPARγ ligand (Fluormone™ PPARγ Green). A 384 well plate was used and compounds tested were first dissolved in DMSO and then dosed into each well with a final volume of 40μL containing 38 nM PPAR γ LBD and 1.25 nM PPAR-Green. A SpectraMax M5 plate reader was used in polarization mode with 485 nm excitation and 535 nm emission. The tested compounds included several major PBDE congeners (e.g., BDE47 and BDE99) and their metabolites [i.e., hydroxylated BDEs (OH-BDEs) and halogenated phenols], halogenated bisphenol A [tetrabromobisphenol A (TBBPA) and tetrachlorobisphenol A (TCBPA)], tris-isopropylpenyl phosphate (ITP), FM550 and related components [i.e., triphenyl phosphate (TPP), 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB) and bis(2-ethylhexyl)-tetrabromophthalate (TBPH)] as well as three major metabolites [i.e., diphenol phosphate (DPP), tetrabromo benzoic acid (TBBA) and tetrabromo mono(2-ethylhexyl)phthalate (TB-MEHP)]. Rosiglitazone was used as positive control for each batch of assay. IC_{50} values and dissociation constants were calculated to compare the potency of binding. These values were calculated according to the following equation:

IC_{50}/[PPARγ Green] = $K_{d,ligand}/K_{d,probe}$, where $K_{d,probe}$ is the dissociation constant calculated from titration of 1.25 nM PPARγ Green with added PPARγ-LBD concentration.

Extracts of dust samples (n = 12) collected from our previous studies were also tested for ligand binding potential. Six of them were US house dust samples, which were collected during 2009. Three of them were

collected in offices in Boston, MA in 2008, and the other three were collected in a gym containing flame retardant foam in 2011-2012. All dust samples were extracted with dichloromethane using sonication. Since a fluorescence polarization (FP) background from the dust matrix was observed, the dust extracts were first cleaned using gel permeation chromatography prior to dosing, collecting elutions between 12 and 26 mins. To minimize the FP background, a single concentration of 3 mg dust/mL in the final 40μL incubation solution was conducted to qualitatively investigate the binding potency of the various dust samples to PPAR γ.

Results and discussion

The calculated IC_{50} values and dissociation constants of the tested compounds with PPARγ-LBD varied considerably. We found that many of these contaminants can competitively bind with PPARγ LBD in a dose-response manner. For example, Firemaster 550 (FM 550) is a mixture of TPP, ITP, TBB and TBPH. As seen in Figure 1, TPP (IC_{50}: 38,000 nM) was found to be a potent ligand of PPARγ and TPP is likely the major contributor to the binding potential of FM550 (IC_{50}: 400,000 nM) to PPARγ, in which TPP accounts for ~20%. The IC_{50} of ITP (60,000 nM), containing ~40% TPP in the mixture, showed a similar dose-response curve with TPP, indicating TPP was an important PPARγ active component in the ITP mixture; however, the other isopropylpenyl phosphate isomers in this mixture may also effectively bind to PPARγ-LBD. And while TBB and TBPH did not show any binding with PPARγ, their their metabolites TBBA and TBMEHP, respectively, can bind to PPARγ. The binding of TBMEHP was particularly potent with an IC_{50} of 640 nM, which was lower than that of one well known PPARγ agonist mono (2-ethylhexyl)phthalate (MEHP) and comparable to PPARγ targeted pharmaceutical compound rosiglitazone (IC_{50}: 230 nM). A clear dose-response was observed for BDE47, but not for BDE99, which was consistent with a previous study using human Osteosarcoma (U2OS) cells-based reporter gene assays[6]. And for the first time, OH-BDEs were found to be potent ligands of PPARγ. 3-OH-BDE47 (IC_{50}: 240 nM) showed a similar binding capacity with that of rosiglitazone, the positive control. The variable IC_{50} values of OH-BDEs also suggest that the position of the hydroxyl group and the number of bromine substituents affect binding to PPARγ. Halogenated phenols were also found to be likely PPARγ ligands and triidiophenol showed the lowest IC_{50} (1840 nM), indicating non-specific hydrophobic interactions with the binding site might favor binding. A similar trend was observed in TCBPA and TBBPA which were potent PPARγ ligands, but binding was not observed for BPA, suggesting that halogenation increases the binding potential.

As to the environmentally relevant samples, binding activity of the dust samples with a concentration of 3mg/mL were observed for 11 out of 12 dust samples tested. As shown in Figure 2, the FP value ranged from 169 to 99 mP with a GM of 126 mP, which was significantly lower than that of the DMSO control (170 mP). The USEPA estimates that children ingest between 50-100 mg/dust day. Therefore, our data demonstrates that environmentally relevant dust exposures have the capacity to significantly interact with PPARγ in vivo. Further work is needed to determine which components in the dust samples are acting as ligands.

In conclusion, this study showed that FRs and their metabolites were potent PPARγ ligands, which might contribute to adipogenesis and possibly exacerbate the obesity epidemic. Furthermore, the binding activity of dust samples was also observed with a high frequency. Therefore, further screening of environmental obesogens in dust samples is needed.

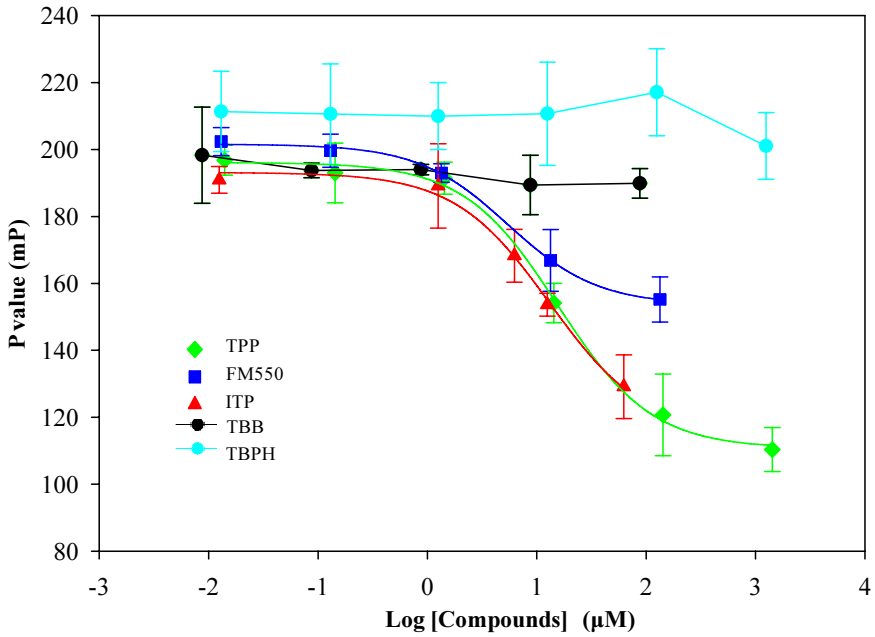

Figure 1. Fluorescence polarization value (mP) as a function of increasing concentrations of FM550, ITP, TPP, TBB, and TBPH in 40µL of 38 nM PPAR ɣ LBD and 1.25 nM PPAR-Green.

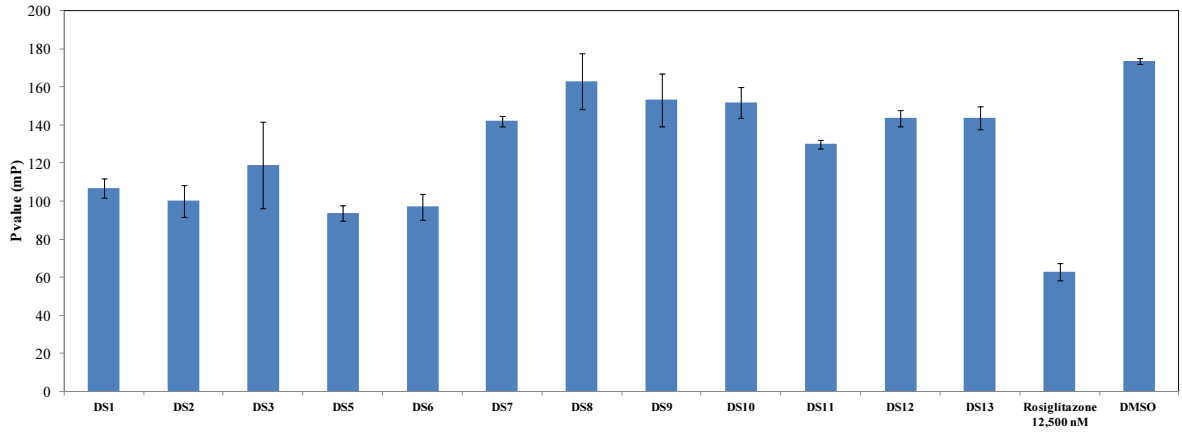

Figure 2. Fluorescence polarization value (mP) of 12 dust samples with a concentration of 3 mg dust/mL in 40µL of 38 nM PPAR ɣ LBD and 1.25 nM PPAR-Green. 12,500 nM rosiglitazone was used as positive control.

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