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Effect of polybrominated diphenyl ether congeners on placental cytokine production

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ABSTRACT

Polybrominated diphenyl ethers (PBDEs) are pollutants that may increase the risk of preterm birth. In previous studies, we found that a mixture of PBDEs altered the expression of biomarkers for preterm birth by the placenta. However, there are 209 different PBDE congeners with different tissue distributions. How these different congeners may alter the production of immunomodulators by the placenta that help to maintain the survival of the fetal allograft is unclear. Therefore, we compared the effects 5 common congeners on basal and bacteria-stimulated cytokine production by the placenta. Placental explant cultures were incubated with 20 μM of PBDE congeners 47, 99, 100, 153, 209 or vehicle in the presence and absence of *Escherichia coli* for 20 h. Conditioned medium was harvested and concentrations of IL-1β, TNF-α, IL-6, sgp130, HO-1, IL-10, BDNF, and 8-IsoP quantified. For unstimulated cultures, all congeners, except for PBDE-47, reduced the production of IL-1β and IL-6 production was enhanced by PBDE-153. BDNF concentrations tended to be reduced by most PBDE congeners and IL-10 production was enhanced by PBDE-99, −153, and −209. 8-IsoP production was enhanced by PBDE-153, but not the other congeners. For bacteria-stimulated cultures, PBDE-47 increased IL-1β production and PBDE-47, −153, and −209 tended to reduce TNF-α production. IL-6 production was enhanced by all PBDEs except 153. IL-10 production was enhanced by all congeners except for PBDE-47. All congeners significantly enhanced BDNF and 8-IsoP. These results suggest that PBDEs can alter the expression of placental biomarkers in a congener and infection-dependent manner.

1. Introduction

Polybrominated diphenyl ethers (PBDE) are the most widespread of the persistent organic pollutants and nearly 100% of women have some level of exposure during pregnancy (Guvenius et al., 2003; Bradman et al., 2007). North American women have much greater exposures to these chemicals where they have been used to satisfy fire and consumer product codes (Bradman et al., 2007). In one study of breast-milk samples, women in the United States had almost 100-fold greater concentrations of PBDE-47, than samples collected from women in Sweden (Schecter et al., 2003), where flame retardants have been banned. In a sampling of immigrant women, concentrations of flame retardants in the plasma were positively correlated with length of residency in the United States (Bradman et al., 2007).

Most studies to date have focused on how these compounds may function as endocrine disruptors of the hypothalamic-pituitary-thyroid axis given their structural similarity to thyroxine and tri-iodothyronine. However, their effects on neurodevelopment and other pregnancy outcomes is unclear. Recent studies have shown that high levels of PBDE exposure in pregnancy are correlated with increased risk for low birth weight (Harley et al., 2011), preterm birth (Peltier et al., 2015), lower verbal and full-scale IQ (Eskenazi et al., 2013), poorer motor control of non-dominant hand (Eskenazi et al., 2013) and increased autistic-like behaviors in the offspring (Braun et al., 2014). Neurodevelopmental disorders may also result from in part from placental dysfunction (Hsiao and Patterson 2011). Changes in the production of inflammatory mediators by the placenta have previously been observed in cultures treated with a mixture of PBDEs (Peltier et al., 2012). Most of the effects of the flame retardants in our previous study were dependent upon bacterial co-stimulation of the cultures (Peltier et al.,

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2012), suggesting that PBDEs may function as risk modifiers, enhancing the inflammatory response to bacteria and possibly other pathogens. We have found similar effects with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as an inflammation enhancer (Peltier et al., 2013) and others have demonstrated that TCDD exposure lowers the dose for bacteria to cause preterm birth in mice (Bruner-Tran and Osteen, 2011). This suggests that it is necessary to study how environmental toxins affect placental immunity in a setting of mild inflammation as well as basal conditions.

Flame retardants exist as 209 different congeners, each consisting of 2 phenyl rings separated by an ether bond but differing in their bromination patterns. The congeners have different tissue distributions with PBDE-47, −99, and −100 being the most abundant in the peripheral plasma (Guvenius et al., 2003) and congeners −153 and −209 being most abundant in the placenta (Frederiksen et al., 2009; Zhao et al., 2013). It is unclear how these different congeners may alter the production of immunomodulators by the placenta that help to maintain the survival of the fetal allograft. Therefore, we compared the effects of PBDE-47, −99, −100, −153, and −209 on basal and bacteria-stimulated cytokine production as well as on biomarkers by placental explant cultures. We chose to evaluate: Interleukin-1β and Tumor Necrosis Factor-α (IL-1β and TNF-α), because they are known proinflammatory cytokines whose dysregulation increases the risk of preterm birth and other adverse pregnancy outcomes; IL-10, because it is produced by the placenta and can inhibit infection-mediated preterm birth; IL-6, because it is a pleiotrophic cytokine whose dysregulation in pregnancy causes neurodevelopmental disorders in animal models; Soluble glycoprotein 130 (sgp130), because it is a negative regulator of IL-6; Heme oxidase-1 (HO-1) because it is produced in response to oxidative stress and inhibits inflammation in the placenta by catalyzing the production of carbon monoxide; 8-Isoprostane (8-IsoP), because it is recognized as one of the best in vivo markers of oxidative stress; and Brain-Derived Neurotrophic Factor (BDNF) because it produced by the placenta, regulates the development of dopaminergic neurons and its production is dysregulated in a number of neurodevelopmental disorders.

2. Materials and methods

The methods for this study are standard for our laboratory and details have been published in previous papers (Peltier et al., 2012; Peltier et al., 2013). All tissue collections were performed with the oversight of the NYU-Winthrop Institutional Review Board.

2.1. Materials

PBDE congeners 47, 99, 100, 153, and 209 were purchased from Accustandard (New Haven, CT) as custom orders to be prepared in dimethyl sulfoxide (DMSO). These congeners were selected because they are the most frequently isolated from normal human placental tissues and/or plasma (Guvenius et al., 2003; Frederiksen et al., 2009; Zhao et al., 2013) and are the major components of commercial flame retardant products. Cultureware and all other chemicals were purchased from Sigma Chemical Company (St. Louis, MO), unless otherwise specified. Heat-killed stocks of *E. coli* were prepared from low-passage frozen stocks of live *E. coli* (ATCC #33908, strain J5) that were initially purchased from the American Type Tissue Collection (Manassas, VA). Bacteria were cultivated in 2 liters of tryptic soy broth and harvested by centrifugation at 10,000*g* for 30 min at 4 °C. Organisms were washed and resuspended in culture medium (RPMI 1640 + 10% fetal bovine serum + 100 μg/ml streptomycin + 100 U/ml Penicillin). Quantitative subcultures were then established to estimate the CFU of the stock bacteria suspension. Organisms were then killed by heating to 80 °C for 1 h, aliquoted and then stored at −80 °C until use in the experiments. Randomly selected stocks were cultured on nutrient agar to confirm that the preparation of aliquoted heat-killed bacteria, estimated to be 10^9 CFU/ml, contained no live bacteria.

2.2. Placental explant cultures

Placental tissues from women who were undergoing elective Cesarean section at term, but who were not in labor, were transported to the laboratory for immediate processing. Tissues were washed extensively with sterile saline and dried blood clots were removed by gentle blotting with sterile gauze. Segments of villous tissues (1–2 cm^3) were isolated from various sites of the placenta by sharp dissection and washed extensively with DMEM in 50 ml conical tubes. Tissues were then minced using an automated tissue chopper (McIlwain model, Mickle Laboratory and Engineering Co., Surry, UK), set to cut at 100 μm increments for 3–5 passes. Chopped tissues were then washed in DMEM and 0.1 g were added to each well of 12-well culture plates. Placental explants were then gently dispersed in tissue culture medium (DMEM + 5% Fetal Bovine Serum, 100 U/ml Penicillin 100 μg/ml Streptomycin) and PBDE congeners and *E. coli* were added to final concentrations of 20 μM (from 100X stocks prepared in complete medium with DMSO) and 10^7 CFU/ml. In this experimental design (a randomized block design), each patient serves as her own control-greatly reducing experimental variation (Fig. 1). Cultures were incubated for 20 h at 37 °C in a humidified incubator under 5% CO_2. Conditioned medium (1 ml) was harvested and stored at −80 °C until assay. Separate sets of placentas were used for unstimulated (n = 10 patients) and *E. coli*-stimulated placentas (n = 12 patients). Effects of PBDE on viability of the tissues was ascertained in a separate set of cultures (n = 4 patients) receiving the same PBDE and bacteria treatments using a variant of the MTT assay as previously described (Peltier et al., 2013).

2.3. Immunoassays

Concentrations of biomarkers for inflammation (IL-1β, TNF-α, IL-6), regulators of inflammation (IL-10, HO-1, sgp130), neurodevelopment (BDNF), and oxidative stress (8-IsoP, HO-1) were quantified using immunoassays purchased from eBiosciences (San Diego, CA), Cayman Chemical (Ann Arbor, MI), Enzo (Farmingdale, NY) or R & D Systems (Minneapolis, MN). Biomarkers whose concentrations were estimated to be below the sensitivity of the assay were set equal to the sensitivity of the assay for that sample. Samples for which biomarker concentrations were above the range of the standard curve were diluted and reassayed until they fell on the curve. All samples were assayed in duplicate and averaged for statistical analysis.

2.4. Statistics

Differences in biomarker production by unstimulated (N = 10) and *E. coli*-stimulated (N = 12) placentas were compared using general linear models. Potential effects of PBDE-treatment on unstimulated as well as bacteria-stimulated cultures were evaluated using linear mixed effects models with the lme4 library of R (http://www.r-project.org). Effects due to bacteria or PBDE treatment were considered fixed and effects due to patient were considered random with a separate intercept for each patient. Fit models were evaluated for assumptions of parametric statistical techniques (normality, independence and equality of errors). Data were log-transformed and reanalyzed whenever these assumptions were not met. For one analysis, data were still not in compliance with the assumptions required for linear mixed effects models and a parametric bootstrap was employed. Results are presented as differences (or fold-difference for log-transformed data) from control ± 95% confidence intervals and considered statistically significant at $P \leq 0.05$. Point estimates (least-squares means ± standard errors) for concentrations of the different biomarkers at each experimental treatment are also provided in supplementary Tables 1 and 2.