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2,4,6-TRIBROMOPHENOL AND POLYBROMINATED DIPHENYL ETHERS (PBDES) IN HUMAN PLACENTAL TISSUES AND THEIR ASSOCIATIONS WITH THYROID HORMONES, THYROID DEIODINASE AND THYROID SULFOTRANSFERASE ACTIVITIES

H. Stapleton[1], C. Leonetti[1], C. Butt[1], K. Hoffman[1], M.L. Miranda[2]
[1]*Duke University, Nicholas School of the Environment, Durham, NC USA*
[2]*Rice University, Department of Statistics, Houston, TX, USA*

Introduction

Brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs) and 2,4,6-tribromophenol (2,4,6-TBP) have been applied to numerous types of furniture and electronic items in order to meet state and federal flammability standards[1]. However, PBDE mixtures were phased out of use and production in the US because of their persistence, bioaccumulation, and potential toxicity beginning in 2004 [2,3]. Despite the phase out, many older products containing PBDEs will remain in use in the home environment, and thus human exposure to PBDEs will continue for some time. In contrast, 2,4,6-TBP is still used today as a flame retardant and as an intermediate in the production of flame retardants. It also has natural sources in the marine environment.

PBDEs and 2,4,6-TBP have chemical structures similar to thyroid hormones (THs), and several studies have demonstrated that they can impact thyroid regulation. One proposed mechanism of TH dysfunction is the disruption of TH-metabolizing enzymes such as deiodinase (DIO) and sulfotransferase (SULT) [4,5]. Disruption of TH metabolism can lead to alterations in serum and/or tissue concentrations of these hormones, which may in turn lead to downstream effects on TH-mediated processes, especially early neurodevelopmental pathways in developing children6. Several epidemiological studies have observed significant associations between maternal and/or cord sera PBDE concentrations and various negative developmental, behavioral, and cognitive effects in young children [7–11].

The developing fetus is exposed to PBDEs throughout gestation by transplacental transport between the maternal and fetal circulatory compartments[12]. During pregnancy, the placenta functions as a regulatory transport barrier for a wide variety of biomolecules[13]. The placenta also facilitates the uptake of THs from maternal circulation and subsequent delivery to the fetal compartment throughout gestation as the fetus does not begin producing its own THs until the beginning of the second trimester and relies solely on maternally-derived THs during the first trimester[14]. During this period, the fetus is sensitive to perturbations in TH homeostasis, and disruption of TH regulation can lead to effects that may manifest later in life as cognitive and/or behavioral deficits[15]. The primary goal of this study was to examine the accumulation of PBDEs and 246- TBP in human placental samples and determine if there were associations with TH concentrations, and measures of TH metabolic enzyme activity, in placental tissues.

Materials and methods

Participants were recruited within an ongoing observational prospective cohort study assessing the joint effect of social, environmental, and host factors on pregnancy outcomes (the Healthy Pregnancy, Healthy Baby (HPHB) Study). The HPHB study enrolled pregnant women from the Duke Obstetrics Clinic and the Durham County Health Department Prenatal Clinic. Our analyses included a subset of women from the HPHB study that delivered at the Duke University Medical Center between March 2010 and December 2011.

Placenta tissue subsamples were taken at the time of delivery at the Duke University Medical Center. Tissues (approximately 5-20 g) were stored in screwtop cryovials at -80°C until analysis. BFRs were measured using previously described methods[17]. All sample values were blank subtracted and MDLs were calculated as three times the standard deviation of the lab blank values for each analyte. TH hormones (T4, T3 and rT3) were measured in placental tissues using a modified version of a previously published method[18].

Preparation of placental microsome fractions and DIO Type 3 assays were performed using a modified method from our laboratory19. Preparation of placental cytosolic fractions and 3,3'-T2 and T3 SULT assays were performed using a modified method from our laboratory [4].

Because we anticipate that gestational age may be related to TH concentrations in placenta, we restricted our analyses to women giving birth to term infants (37 or more weeks gestation (n=95)). Preliminary analyses (Shapiro-Wilkes Test) indicated that TH, BFR, and enzyme activity data were not normally distributed. Accordingly, non-parametric statistical tests were used or data were log10 transformed prior to statistical analyses. We used Spearman correlation coefficients to assess associations between BFRs, THs, and DIO and SULT activities. To assess factors associated with placenta BFRs and relationships between BFRs and THs/DIO/SULT, we used linear regression models (continuous outcome measures were log10-transformed). To aid in the interpretation of regression results, we exponentiated beta coefficients (10^{β}), producing the multiplicative change in outcome. As predictors of THs and enzyme activity, BFR analytes were categorized into tertiles in order to minimize the effect of skewed data and outliers. Analyses were also adjusted for maternal age and gestational age at delivery, factors which we anticipated might confound associations between BFRs and THs. Additionally, we hypothesize that there may be important sex difference in associations between BFRs and THs. To explore these differences, we conducted all analyses in the full cohort (combined group), but also conducted analyses stratified by the sex of the infant.

Results and discussion

Detailed information on the concentrations of different PBDE congeners and 2,4,6-TBP in placental tissues are reported in Leonetti et al. 2016[17]. Briefly, detection frequencies for BDE-47, -100, -99, -154, -153, -209, and 2,4,6-TBP were all > 50%. The most abundant PBDE measured was BDE-47, representing 34% of ΣBDE burden. The geometric mean concentration of 2,4,6-TBP was 15.4 ng/g lipid (range: 1.31 - 316 ng/g lipid), while the geometric mean concentration of BDE-47 was 5.09 ng/g lipid (range: 0.12 - 141 ng/g lipid). Interestingly, concentrations were higher in placental tissues associated with male infants compared to female infants and were statistically significant for BDE-209, 2,4,6-TBP, and ΣBDE ($p = 0.002$, 0.004, and 0.008, respectively).

In analyses including all placental tissues, BFRs were not correlated with placental T3 or T4 concentrations. However, among females, a statistically significant positive association was observed between T3 and BDE-99, 2,4,6-TBP, and ΣBFRs, while BDE-47, -153, and ΣBDEs showed a suggestive positive association with T3. Concentrations of rT3 in placental tissue were significantly inversely correlated with BDE-99 ($rs = -0.34$; $p=0.02$) for both males and females, and in males, an inverse association was observed between rT3 and BDE-209 ($rs = -0.35$; $p = 0.01$).

To provide additional information, adjusted regression analyses were conducted. As in the correlation analyses, none of the BFRs were significantly associated with T3 in analyses including all participants. However, although results generally did not reach statistically significance, BDE-47, -99, and -100 were negatively associated with T3 concentrations among males, while BDE-47, -99, -100, -153 and 2,4,6-TBP were positively associated with T3 among females. For example, among males, those with the highest concentrations of BDE-99 in placenta had T3 levels 0.80 times those with the lowest concentration of BDE-99 (95% confidence interval (CI): 0.59, 1.07). Whereas females with the highest concentrations of BDE-99 in placenta had T3 levels 1.50 times those with the lowest concentration of BDE-99 (95% CI: 1.10, 2.04). We observed little evidence of association between any of the BFR analytes and T4 concentrations.

DIO3 activity was measured in all placenta tissue microsome samples. In regression analyses, DIO3 activity among males showed a suggestive positive association with BDE-99, -100, and -153, however, no relationships were statistically significant. In females, DIO3 activity showed a suggestive negative association in the highest tertile for BDE-47, -99, -100, -153, and 2,4,6-TBP, with BDE-99 having the strongest association and was statistically significant ($10^{\beta} = 0.49$, CI: 0.26, 0.91).

TH SULT activity was measured in all placenta tissue cytosol samples. In adjusted regression models T3 SULT activity among males was positively associated with BDE-47, -100, and -153, with BDE-153 having the strongest association ($10^{\beta} = 1.48$, CI: 1.05, 2.09 comparing the 3rd to 1st tertile). For females, T3S activity showed a negative association with BDE-99 ($10^{\beta} = 0.67$, CI: 0.49, 0.91).

These results suggest there may be sex-specific differences in PBDE effects on placental TH regulation. Sex-specific effects have been previously observed for various serum perflouroalkyl substances (PFASs) which were differentially associated with TH levels [20,21]. These studies are similar to our current results in that we observed a negative trend for THs in males, and a positive trend for THs in females. Although these are different classes of chemicals, BFRs and PFASs share some similar endocrine disrupting capacities and have both been shown to impact the HPT axis. The mechanisms underlying the sex-specific effects of TH disruption are unknown; however, it is known that sex hormones can modulate TH levels[22]. Additional sex differences have been observed in animal studies, in which the biological half-lives of some chemicals were found to be up to 70 times longer in males versus females[23–25]. Interestingly, the concentrations of all BFR analytes are higher in placenta tissues from male infants than in placenta tissue from female infants in this study. These levels are significantly higher in males compared to females for BDE-209, and 2,4,6-TBP, by approximately a two-fold difference. While it is possible that differences in the lipid content of the placenta tissues can affect the PBDE levels reported, we observed no significant differences in lipid content of the placenta tissue samples by infant sex.

The underlying mechanism of this sex-specific difference in PBDEs associations is not known at this time, however, the sex of the infant seems to play a role in the bioaccumulation potential, thyroid hormone metabolism, and/or kinetic parameters of the placenta during pregnancy. It is possible that the sex-specific differences in placental morphology and structure may play a role in this organ's ability to transfer and metabolize BFRs [26]. Further studies are needed to verify these results and investigate mechanisms and health outcomes in infants.

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