Environment International 112 (2018) 269–278

Contents lists available at ScienceDirect

Environment International

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

Polybrominated diphenyl ethers (PBDEs) and hydroxylated PBDE metabolites (OH-PBDEs) in maternal and fetal tissues, and associations with fetal cytochrome P450 gene expression

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ARTICLE INFO

Keywords:
Brominated flame retardants
Fetal metabolism
Prenatal exposures
OH-PBDEs
Endocrine disrupters
Cytochrome P-450 enzymes
Placenta transport
Organohalogen chemicals

ABSTRACT

Background: Human fetal exposures to polybrominated diphenyl ethers (PBDEs) and their metabolites (OH-PBDEs) are unique from adults, and in combination with a different metabolic profile, may make fetal development more sensitive to adverse health outcomes from these exposures. However, we lack data to characterize human fetal PBDE exposures and the metabolic factors that can influence these exposures.
Objective: We examined differences between 2nd trimester maternal and fetal exposures to PBDEs and OH-PBDEs. We also characterized fetal cytochrome P450 (CYP) mRNA expression and its associations with PBDE exposures.
Methods: We collected paired samples of maternal serum and fetal liver (n = 86) with a subset having matched placenta (n = 50). We measured PBDEs, OH-PBDEs, and mRNA expression of CYP genes (e.g. CYP1A1, -2E1, -2J2, -2C9) in all samples. As a sensitivity analysis, we measured PBDEs and OH-PBDEs in umbilical cord serum from a subset (n = 22).
Results: BDE-47 was detected in ≥ 96% of all tissues. Unadjusted ΣPBDEs concentrations were highest in fetal liver (geometric mean (GM) = 0.72 ng/g), whereas lipid-adjusted concentrations were highest in cord serum (111.12 ng/g lipid). In both cases, fetal concentrations were approximately two times higher than maternal serum levels (GM = 0.33 ng/g or 48.75 ng/g lipid). ΣOH-PBDEs were highest in maternal and cord sera and 20–200 times lower than PBDE concentrations. In regression models, maternal BDE-47 explained more of the model variance of liver than of placenta BDE-47 concentrations (adjusted R^2 = 0.79 vs 0.48, respectively). In adjusted logistic regression models, ΣPBDEs were positively associated with expression of CYP2E1 and -2J2 (placenta), and -1A1 (liver) ($p < 0.05$).
Conclusion: Our findings suggest that under normal conditions of mid-gestation, the human fetus is directly exposed to concentrations of PBDEs that may be higher than previously estimated based on maternal serum and that these exposures are associated with the expression of mRNAs coding for CYP enzymes. These results will help frame and interpret findings from studies that use maternal or cord blood as proxy measures of fetal exposures, and will inform the molecular pathways by which PBDEs affect human health.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are persistent organic pollutants that have been widely used as flame retardants in consumer products since the 1970s. Although use of PBDEs is being phased out, their ongoing presence in durable consumer products, in food, and in indoor dust suggest that humans will continue to be exposed to PBDEs and these exposures will continue to bioaccumulate (Frederiksen et al., 2009; Zota et al., 2013). Consequently, people worldwide will remain exposed to these chemicals for decades (Mitro et al., 2015).

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https://doi.org/10.1016/j.envint.2017.12.030
Received 31 July 2017; Received in revised form 18 December 2017; Accepted 19 December 2017

Biomonitoring studies show that > 90% of pregnant women are exposed to at least one of the PBDE congeners (Woodruff et al., 2011). Developmental PBDE exposure is associated with cognitive deficits in children (e.g., (Lam et al., 2017)). These studies report that developmental exposure to PBDEs is associated with mental and physical development (Herbstman et al., 2010), Full-Scale Intelligence Quotient, reading skills and externalizing behaviors (Vuong et al., 2017a; Zhang et al., 2017), executive function (Vuong et al., 2017b) and other neurobehaviors (Lam et al., 2017). Some of these reports focus on prenatal exposure by measuring PBDEs in maternal and/or cord blood, while others focus on post-natal exposures by measuring PBDE exposures in children's blood (e.g., (Vuong et al., 2017c)).

While these studies provide sufficient evidence to support an association of PBDE exposure and cognitive deficits in children, there are important uncertainties. Specifically, it is not clear that PBDE congeners and/or the metabolites that prenatally mediate neurobehavioral effects actually cross the placenta to reach the fetus. Although maternal exposure to PBDEs may affect maternal or placental physiology such that adverse effects are observed in the offspring, it is important to determine whether PBDEs and/or their metabolites could also exert adverse effects by acting directly on the fetus. However, we have limited information about fetal exposures to PBDEs that occur over the course of pregnancy or to target organs of concern. One study demonstrates that PBDEs can be measured in human fetal liver (Schecter et al., 2007). However, these fetuses were stillborn; thus, their pathology may have contributed to placental passage of PBDEs. Moreover, PBDEs were not measured in maternal serum, thereby making it difficult to determine the relationship between maternal and fetal exposures.

There are also existing data gaps on fetal metabolism of PBDEs, which is critical to evaluating developmental PBDE toxicity since PBDE metabolites may be more biologically active than parent PBDEs within some domains (e.g., (Stapleton et al., 2011)). Cytochrome (CYP) p450 enzymes metabolize both xenobiotic and endogenous substances and may be critical to PBDE-mediated toxicity. In vitro, CYPs such as CYP2B6, metabolize PBDEs to form hydroxylated metabolites (OH-PBDEs) (Erratico et al., 2012). Additionally, PBDEs may alter CYP expression in adult human liver cells (Stapleton et al., 2009). However, much of the data on CYPs is based on studies of adult human cells and rodent studies, and CYP levels and activity will not be the same during human fetal development due to the age-dependent expression of CYPs in the fetal liver and unique contributions of placental CYP expression (Hakkola et al., 1998). There is evidence in rats that perinatal exposures to BDE-47 or -99 can increase expression of CYP enzymes in the fetal and postnatal liver (Blanco et al., 2012; Suvorov and Takser, 2010), but we do not have similar data in humans.

Therefore, the goal of this study was to evaluate maternal/fetal transfer of PBDEs and their metabolites during the second trimester of pregnancy, a period of rapid biologic development that can result in increased sensitivity to environmental stressors. To accomplish this, we measured several PBDEs and their metabolites in normal fetal liver, placenta, and maternal and cord sera. In addition, we measured the expression of several mRNAs coding for CYP enzymes to identify those that may be related to PBDE exposure and metabolism. We focus on mRNAs coding for CYP enzymes, rather than CYP enzyme activity, because environmental chemicals can induce the transcriptional activity of genes coding for these enzymes; therefore, mRNA levels are more directly related to induction by environmental factors.

2. Materials and methods

2.1. Study population

In 2011–2012 and 2014, we recruited and consented English- and Spanish-speaking patients between 15 and 24 weeks of pregnancy seeking medical care from the University of California, San Francisco (UCSF) Women's Options Center at San Francisco General Hospital in San Francisco, California. The Women's Options Center is an outpatient clinic providing pregnancy terminations and serving an ethnically diverse and predominantly lower income population from the San Francisco Bay Area and other parts of California. Eligible study participants were identified by reviewing the patient's medical record only after they had 1) consulted with a trained counselor for an elective second trimester termination procedure and 2) consented to the procedure as documentation of intent to proceed with the elective pregnancy termination. The 2011–2012 cohort (Cohort 1) consists of 36 smoking and non-smoking women and the 2014 cohort (Cohort 2) consists of 50 former or non-smoking women. For both cohorts, we excluded participants if they were seeking a termination because of fetal anomalies. All study protocols were approved by the UCSF Committee on Human Research.

2.2. Sample collection and preparation

Maternal blood and fetal liver samples were collected in both study populations. Placenta was collected in Cohort 2 ($n = 50$), and as a sensitivity analysis, cord blood was collected in a subset of Cohort 1 ($n = 22$). Maternal blood was collected from each participant prior to medical procedures in red top Vacutainer tubes. The time between blood collection and prior food or fluid consumption ranged from 0.5–23 h (mean = 13 h; $n = 65$). Women's Options Center medical staff collected umbilical cord blood with assistance from our study team. Umbilical cord blood was drained directly into red top collection tubes during the procedure to avoid contact with medical devices and the environment. However, minimal contamination from maternal blood on the umbilical cord could not be precluded. After collection, both the maternal and umbilical cord blood samples were centrifuged at 3000 RPM for 10 min at 4 °C. Serum was aliquoted using glass pipettes into sterilized amber vials, which were pre-screened for environmental contamination, and stored at − 80 °C until analysis. At the end of the dilation and evacuation procedure, research assistants collected samples of placenta and fetal liver for chemical and RNA expression analyses using dissecting forceps. The samples collected for chemical analyses were washed in ice-cold phosphate-buffer solution (PBS) and then stored in pre-screened amber jars at − 80 °C. In Cohort 1, tissues collected for RNA expression analyses were washed in cold PBS, soaked and incubated in RNAlater solution in RNAlater Tissue Protector Tubes (2–8 °C, overnight), and stored at − 80 °C. Tissues for RNA analyses in Cohort 2 were washed in cold PBS, flash frozen, and stored at − 80 °C.

2.3. Environmental chemical analysis

We analyzed 19 PBDE congeners and eight OH-PBDEs at the Department of Toxic Substances Control (DTSC) (Berkeley, CA, USA) within its clean laboratory facility, where human specimens are exclusively processed. In this study, we focus our analysis on the congeners detected in > 50% in maternal serum (BDE-28, -47, -99, -100, -153) and the four most commonly detected OH-PBDEs (5-OHBDE-47, 6-OHBDE-47, 5-OHBDE-99, 4-OHBDE-49) in maternal serum, fetal liver, and placenta. We also present preliminary data on a smaller subset of cord blood samples.

Liquid-liquid extraction and phase separation were used to separate the phenolic compounds from neutral compounds and are described in detail elsewhere (Zota et al., 2011). Initial sample preparation steps differed depending on the matrix. Briefly, placenta was lyophilized and homogenized with sterile scalpels and a 10 min sonication was added during the protein denaturalization and liquid-liquid extraction step to maximize extraction efficiency. Fetal liver, once homogenized with a scalpel, behaved similarly to serum and was extracted in the same manner. After liquid-liquid extraction, placenta and liver extracts were measured for lipid content. Re-suspension and phase separation of phenolic compounds followed.

PBDEs and OH-PBDEs were analyzed in separate sample aliquots.