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PBDE METABOLISM TO OH-BDES: INVESTIGATION OF CYP 2B6 MECHANISTIC VARIABILITY

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Introduction

The presence of hydroxylated brominated diphenyl ethers (OH-BDEs) in adults and children have been a concern since the early 2000s based on their ubiquitous detection. OH-BDEs are known to be biotransformation products of the flame retardants, polybrominated diphenyl ethers (PBDEs), but are also present naturally in the environment. Due to differences in exposure routes, concentrations of OH-BDEs have been detected at greater levels in children when compared to adults [1-3], which can be detrimental for developing infants due to the neurotoxic endpoints of these compounds. However, to this day, the fate of OH-BDEs in humans remains unclear.

In vitro studies demonstrated that the three most dominant PBDE congeners, PBDE-47, -99, and -100, are metabolized in the body into OH-BDEs by the cytochrome P450 2B6 (CYP 2B6) enzyme [4]. Yet, metabolism rates vary 10-100 fold between individuals and also among ethnic populations such as Asians, Europeans, Africans, and North Americans [5]. The variation in the rates of metabolism has been suggested to be due to the differences in single nucleotide polymorphisms (SNPs) from amino acid splicing that is altered by CYP 2B6 alleles and need to be further investigated.

The aim of this work is to characterize the genetic variability of CYP 2B6 variants (CYP 2B6*1, CYP 2B6*4, CYP 2B6*5, CYP 2B6*6, and CYP 2B6*7) in regards to the levels of PBDEs and OH-BDEs found in blood serum from a cohort of twenty-four women from the United States. Correlation of these levels will further our understanding of the enzyme specific metabolism of PBDEs to OH-BDEs in humans.

Materials and Methods

Samples

Blood serum from twenty-four female volunteers from Texas, USA was collected for the analysis of PBDEs and OH-BDEs. Buccal cells were collected from each volunteer and DNA was isolated using a QIAmp DNA Mini Kit. The DNA was then genotyped for CYP 2B6 variants *1, *4, *5, *6, and *7 using multiplex polymerase chain reaction (PCR).

Sample extraction and analysis

One gram of serum was spiked with a 13C-labeled surrogate mixture containing 13C-PBDEs and 13C-OH-BDEs for sample recovery. Extraction of targeted analytes via pressurized liquid extraction was used, followed by a two-stage clean up step with acidification to remove interfering lipids. Prior to injection, the OH-BDEs were derivatized using N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) to form OH-TBDMS-BDE derivatives. All PBDEs and OH-TBDMS-BDEs were analyzed in a "one-shot" analysis using gas chromatography tandem mass spectrometry (GC-MS/MS). More details on the extraction and instrument parameters are discussed in a previously published method[6].

Results and Discussion

Concentrations of PBDEs and OH-BDEs

Concentrations of detected PBDEs and OH-BDEs are shown in Table 1 in regards to the expression of the donors' CYP 2B6 variants. PBDE-47, -99, -100, and -153 were detected in almost every serum sample with median levels of 32.6, 11.1, 4.70, and 7.30 ng/g lipid, respectively. Concentrations of OH-BDEs were one magnitude lower than concentrations of PBDEs, and were dominated by OH-tetra-BDEs. The metabolite with the greatest positive detection was 5-OH-BDE-47, followed by 6-OH-BDE-47 and 3-OH-BDE-47, which follows in vitro experimental metabolism rates using human liver microsomes[4].

Characterization of PBDEs and OH-BDEs to CYP 2B6 variants
The four CYP 2B6 variants expressed in the women were CYP 2B6 *1, *5, *6 and *7. However, CYP 2B6*1 (n=12) and CYP 2B6*6 (n=8) were the most dominant genotypes expressed and concentrations of PBDE-47, -99, -100, and -153, and were thus compared to levels of OH-BDEs (Table 2). Women with the CYP 2B6*1 allele showed no correlation among ∑3 OH-BDEs (5-OH-BDE-47, 6-OH-BDE-47, and 3-OH-BDE-47) and any PBDE ($R2<0.170$). However, women with the CYP 2B6*6 variant observed high correlation with PBDE-47, -99, and -100 with ∑3 OH-BDEs ($R2>0.780$). Given that the characterization of PBDEs and OH-BDEs with the CYP 2B6*1 and *6 alleles are greatly varied; it is evident that the metabolism rate and functionality of the CYP 2B6 isoforms can affect pharmacological and toxicological responses in humans.

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