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Disruption of thyroid hormone sulfotransferase activity by brominated flame retardant chemicals in the human choriocarcinoma placenta cell line, BeWo

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HIGHLIGHTS

- Some BFRs significantly inhibited thyroid SULT activity in BeWO cell cultures.
- 2,4,6-tribromophenol displayed the greatest inhibition of SULT activity.
- No effects were observed on thyroid hormone levels in cell culture.
- Mass spectrometry analysis suggests OH-BDEs are likely substrates for SULTs.

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ABSTRACT

Brominated flame retardants (BFRs) have been shown to disrupt thyroid hormone (TH) homeostasis through multiple mechanisms, including inhibition of enzymes that regulate intracellular levels of THs, such as sulfotransferases (SULTs). The placenta plays a critical role in helping to maintain TH levels during fetal development and expresses SULTs. This is concerning given that disruption of TH regulation within the placenta could potentially harm the developing fetus. In this study, we investigated the effects of two polybrominated diphenyl ethers (PBDEs), two hydroxylated PBDEs, and 2,4,6-tribromophenol (2,4,6-TBP) on TH SULT activity in a choriocarcinoma placenta cell line (BeWo). BeWo cells were exposed to BFR concentrations up to 1 μM for 1–24 h to investigate changes in basal SULT activity and in mRNA expression of several TH regulating genes. 2,4,6-TBP was the most potent inhibitor of basal 3,3′-T2 SULT activity at all exposure durations, decreasing activity by as much as 86% after 24 h of exposure. BDE-99, 3-OH BDE-47, and 6-OH BDE-47 also decreased 3,3′-T2 SULT activity by 23–42% at concentrations of 0.5 μM and 1.0 μM following 24 h exposures. BDE-47 had no effect on SULT activity, and there was no observed effect of any BFR exposure on expression of SULT1A1, or thyroid nuclear receptors alpha or beta. This research demonstrates that total TH SULT activity in placental cells are sensitive to BFR exposure; however, the mechanisms and consequences have yet to be fully elucidated.

1. Introduction

Brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs) have been historically used in various consumer products to meet flammability regulations. However, due to their demonstrated toxicity and environmental persistence, the PBDE commercial mixtures PentaBDE and OctaBDE were banned or phased-out, depending on the region, in the mid-2000s, while DecaBDE was phased-out at the end of 2013 (AgencyDecahase, 2009). Despite this phase-out, human exposure to PBDEs remains ubiquitous, and occurs primarily through inadvertent ingestion of contaminated house dust, with infants and toddlers experiencing

Abbreviations: 2,4,6-TBP, 2,4,6-tribromophenol; DI, deiodinase; DIO3, type 3 deiodinase; GC/MS, gas chromatography/mass spectrometry; IC_{50}, half-maximal inhibitory concentration; K_m, Michaelis constant; LC-MS/MS, liquid chromatography-tandem mass spectrometry; OH-BDE, hydroxylated PBDE; PHAH-OHs, polyhalogenated aromatic hydrocarbons; PBDE, polybrominated diphenyl ether; RT-qPCR, quantitative real-time reverse transcription polymerase chain reaction; SULT, sulfotransferase; T4, thyroxine; rT3, 3,3′,5′-triiodothyronine; T3, 3,3′,5-triiodothyronine; 3,3′-T2, 3,3′-diiodothyronine; TH, thyroid hormone; UDPGT, uridine diphosphate glucuronosyltransferase; V_{max}, maximal enzyme velocity.

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higher rates of exposure due to their frequent hand-to-mouth contact (Johnson et al., 2010; Stapleton et al., 2012).

There is growing human epidemiological data suggesting that PBDE exposure may lead to poorer health outcomes, including associations with adverse birth outcomes, neurodevelopmental deficits, difficulties in becoming pregnant, and changes in thyroid hormone levels (Abdelouahab et al., 2013; Harley et al., 2010; Miranda et al., 2015). The human placenta plays a critical role in the delivery of maternally-derived thyroid hormones (THs) to the fetal compartment during pregnancy. This is especially important during the first trimester of pregnancy when the fetus is undergoing many TH-mediated neurodevelopmental processes (Chan et al., 2009). Therefore, gestation represents an extremely vulnerable window of fetal development that is susceptible to disruption by environmental contaminants.

Previous research from our laboratory demonstrated that some BFRs can inhibit thyroid deiodinase (DIO) and thyroid sulfotransferase (SULT) activity in human liver sub-cellular fractions (Butt and Stapleton, 2013; Butt et al., 2011). In particular, human hepatic SULT activity was more sensitive to inhibition compared to DIO, especially for several halogenated BFRs (e.g. hydroxylated PBDEs, OH-BDEs, and 2,4,6-tribromophenol). Recently, we also measured the accumulation of BFRs in archived human placental tissues and examined associations between BFRs, THs, and DIO and SULT activity (Leonetti et al., 2016). These studies suggest that exposure to BFRs may be altering local TH levels in the placenta through DIO and SULT activity. There are eight SULT isozymes that have been shown to perform TH sulfation (SULT1A1, 1A3, 1A5, 1B1, 1B2, 1C1, 1E1, and A1) and SULT1A1 and SULT1A3 are the dominant isoforms present in human placenta (Kester et al., 1999; Stanley et al., 2001). However, given the complexity involved in using human tissues and the numerous confounding variables present in human studies, the use of a more controlled *in vitro* experimental system is needed to provide additional clarity into the underlying mechanisms of action. To this end, this project was undertaken to evaluate the effects of BFR exposure on TH SULT activity using the human placenta cell line, BeWo.

The BeWo cell line is a malignant human choriocarcinoma cell line derived from a methotrexate-resistant tumor (Pattillo et al., 1968). Studies have confirmed the BeWo cell line to be ultrastructurally similar to *in utero* trophoblast cells, indicating that they are an appropriate model for placenta cell culture experiments (Friedman and Skehan, 1979). Additionally, because their cell type composition is dominated by cytotrophoblasts, the BeWo cell line is more similar to an early-stage/first trimester placenta before subsequent differentiation and proliferation of syncytiotrophoblasts. This is important because the early-stage placenta represents the most vulnerable developmental window of exposure for the fetus. Thus, the use of the BeWo cell line can provide insight into this difficult-to-study developmental period.

The goal of this study was to investigate the effects of BFRs on TH SULT activity in the BeWo cell line, as a surrogate for placental tissues. We sought to determine if these specific BFRs will inhibit sulfation of thyroid hormones, and lead to increases in the intracellular concentrations of THs (e.g. triiodothyronine, T3). We also measured gene expression of several SULT isoforms, and thyroid hormone nuclear receptors.

2. Materials and methods

2.1. Reagents and materials

2,4,6-TBP (99% pure), T4 (>99%), T3 (>99%), rT3 (>99%), 3,3′-T2 (>99%), and adenosine 3′- phosphate 5′-phosphosulfate (PAPS) lithium salt hydrate (>60%) were purchased from Sigma-Aldrich (St. Louis, MO). BDE-47 (>97%), BDE-99 (>97%), 3-OH 2,2′,4,4′-BDE-47 (3-OH BDE-47; 99%), and 6-OH 2,2′,4,4′-BDE-47 (6-OH BDE-47; 100%) were purchased as neat standards from AccuStandard (New Haven, CT). [$^{13}C_6$]-3,3′-T2 was purchased from Isotec (Miamisburg, OH). 3,3′-T2 sulfate (3,3′-T2S; 98%) was custom synthesized by the Duke University Small Molecule Synthesis Facility (Durham, NC). T3 sulfate (T3S) was purchased from Toronto Research Chemicals (Toronto, ON; 98.6%).

Ham's F-12K (Kaighn's Modification) Medium cell culture media and other cell culture reagents were purchased from Life Technologies (Carlsbad, CA). All solvents and other reagents were purchased from VWR (Radnor, PA). BeWo cells (ATCC® CCL-98™) were obtained from the Duke University Cell Culture Facility (Durham, NC). All cells were grown in Tissue Culture treated flasks (75 and 182 cm (Johnson et al., 2010) and dishes (36.3 cm^2) from Genesee Scientific (San Diego, CA).

2.2. Cell culture

BeWo cells were grown in Ham's F-12K (Kaighn's Modification) Medium cell culture media supplemented with 10% FBS, 30 nM selenium (as sodium selenite), 100 units mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin at 37 °C and 5% CO_2. All experiments were performed with cells that were thawed from the same passage number, and all replicate experiments were performed with cells from different aliquots of the same source of cells in order to ensure and evaluate reproducibility. Cells were thawed and plated in T-75 flasks, media was changed every 48 h, and following the initial growth period cells were passaged and transferred to T-182 flasks for growth (1:3 passage ratio used; passage at 80% confluency/every 96 h).

2.3. Dosing

Cells were passaged and transferred to cell culture dishes and seeded at a density of 1.0×10^6 cell cm^{-2}. Cells were allowed to grow for 36 h, after which point the growth media was replaced with the appropriate dosing media (cell culture growth media + inhibitor dissolved in DMSO). After the allotted exposure period (1, 6, 12, or 24 h), the growth media was collected and stored for TH analysis and verification of dosing concentrations. Cell culture dishes were rinsed with Dulbecco's Phosphate Buffered Saline without calcium chloride and magnesium chloride. Cell culture dishes were then loaded with 1 mL of phosphate sucrose buffer (KPO_4 buffer containing 0.25 M sucrose and 1 mM EDTA) and cells were stored at −80 °C for future analysis.

The low concentration for 2,4,6-TBP, BDE-47, and BDE-99 was 0.05 μM (50 nM) to approximately reflect the 95th percentile of human serum concentrations measured in the United States (Stapleton et al., 2008a). Similarly, the low concentration selected for 3-OH BDE-47 and 6-OH BDE-47 was 0.001 μM (1 nM) in order to represent levels approximating the 95th percentile of human serum concentrations measured in the United States (Chen et al., 2013). The high and medium concentrations were the same for all BFR compounds tested (1.0 μM and 0.5 μM, respectively). All dosing compounds were dissolved in DMSO at 1000 times higher than the desired final concentration in the cell culture media to ensure a concentration of 0.1% DMSO. The concentrations of the compounds in the dosing media were verified using GC-MS and LC-MS/MS methods (Stapleton et al., 2008b; Erratico et al., 2010).

2.4. Cytotoxicity

Cytotoxicity was assessed using a resazurin cell viability assay