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Protective effect of (±)α-tocopherol on brominated diphenyl ether-47-stimulated prostaglandin pathways in human extravillous trophoblasts *in vitro*

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ABSTRACT

Brominated diphenyl ether (BDE)-47 is a prevalent flame retardant chemical found in human tissues and is linked to adverse pregnancy outcomes in humans. Because dysregulation of the prostaglandin pathway is implicated in adverse pregnancy outcomes, the present study investigates BDE-47 induction of prostaglandin synthesis in a human extravillous trophoblast cell line, HTR-8/SVneo, examining the hypothesis that BDE-47 increases generation of reactive oxygen species (ROS) to stimulate the prostaglandin response. Treatment with 20 μM BDE-47 significantly increased mRNA expression of prostaglandin-endoperoxide synthase 2 (PTGS2) at 4, 12 and 24 h, and 24-h treatment significantly increased cyclooxygenase (COX)-2 cellular protein expression and prostaglandin E2 (PGE2) concentration in culture medium. The BDE-47-stimulated PGE2 release was inhibited by the COX inhibitors indomethacin and NS398, implicating COX activity. Exposure to 20 μM BDE-47 significantly increased ROS generation as measured by carboxydichlorofluorescein fluorescence, and this response was blocked by cotreatment with the peroxyl radical scavenger (±)-α-tocopherol. (±)-α-Tocopherol cotreatment suppressed BDE-47-stimulated increases of PGE2 release without significant effects on COX-2 mRNA and protein expression, implicating a role for ROS in post-translational regulation of COX activity. Because prostaglandins regulate trophoblast functions necessary for placentation and pregnancy, further investigation is warranted of BDE-47 impacts on trophoblast responses.

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1. Introduction

Proper placental development is prerequisite for a successful pregnancy. Abnormal placentation contributes to the pathophysiology of adverse obstetrical complications such as preeclampsia (Brosens, 1977; Gerretsen et al., 1981; Robertson et al., 1967; Sheppard and Bonnar, 1976), intrauterine growth restriction (Gerretsen et al., 1981; Hustin et al., 1983; Labarrere and Althabe, 1987; Sheppard and Bonnar, 1981), spontaneous abortion (Hustin et al., 1990; Khong et al., 1987), preterm premature rupture of membranes (Kim et al., 2002), and preterm birth (Kim et al., 2003). Although the mechanisms responsible for improper placentation have not been fully elucidated, the role of impaired trophoblast invasion has been implicated (Zhou et al., 1997a).

The extravillous trophoblasts (EVTs) are a highly proliferative and migratory cell population that invades the decidual and myometrial segments of the spiral arteries, resulting in the reversible remodeling of the arterial wall architecture (Anton et al., 2012; Brosens et al., 1967; Pijnenborg et al., 1983, 1980). Trophoblast invasion is tightly regulated by a number of autocrine and paracrine factors including growth factors, growth factor-binding proteins, and proteoglycans (Chakraborty et al., 2002; Lala and Chakraborty, 2003). Recently, inflammatory mediators such cytokines and prostaglandins have been shown to play a role in the regulation of trophoblast function during first trimester of pregnancy (Biondi et al., 2006; Horita et al., 2007; Jovanovic et al., 2010; Jovanovic and Vicovac, 2009; Nicola et al., 2005).

Abbreviations: BDE-47, brominated diphenyl ether-47; carboxy-H2DCF-DA, 6-carboxy dichlorodihydrofluorescein diacetate; COX-2, cyclooxygenase-2; cPGES, cytosolic prostaglandin E synthase; DCF, dichlorofluorescein; cDCF, carboxydichlorofluorescein; DMSO, dimethyl sulfoxide; HPGD, gene for 15-hydroxyprostaglandin dehydrogenase; mPGES-1, membrane-bound prostaglandin E synthase-1; mPGES-2, membrane-bound prostaglandin E synthase-2; NO, nitric oxide; ONOO, peroxynitrite; PBDE, polybrominated diphenyl ether; PGE2, prostaglandin E2; PGES, prostaglandin E synthase; PLA2, phospholipase A2; PTGES, gene for prostaglandin E synthase; PTGS2, gene for prostaglandin-endoperoxide synthase 2 or COX-2; ROS, reactive oxygen species; TBHP, *tert*-butyl hydroperoxide; TXB2, thromboxane B2.

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Prostaglandins are small lipid molecules synthesized from membrane phospholipids in response to various physiological and pathological stimuli (Nicola et al., 2005). Of these, prostaglandin E2 (PGE2) is one of the most extensively studied prostaglandins, and has been shown to play critical roles in processes required in successful pregnancy, for example, implantation (Psychoyos et al., 1995; Yee et al., 1993), immunoprotection of the semiallogenic conceptus (Parhar et al., 1988), and parturition (Keelan et al., 2003). Dysregulation of PGE2 production within the gestational compartment has been linked to adverse birth outcomes such as intrauterine growth restriction, preeclampsia and preterm birth (Germain et al., 1999; Ness and Sibai, 2006). Although it is not fully understood how dysregulated prostaglandin pathways lead to these adverse impacts, it is suggested that PGE2 regulates trophoblast cellular functions that are critical for successful placentation (Biondi et al., 2006; Horita et al., 2007; Nicola et al., 2005).

Polybrominated diphenyl ethers (PBDEs) are commercially produced synthetic flame retardants that have been used in textiles, plastics, building materials and insulation (Miller et al., 2009). Because of PBDEs' environmental persistence and toxicity, the US EPA has identified PBDEs as a priority human health concern (U.S. Environmental Protection Agency, 2006). Limited studies report reproductive toxicity of PBDEs during pregnancy. Rabbits orally exposed to PBDEs showed decreased gestation length (Breslin et al., 1989). In human studies, Main et al. reported a significantly higher risk of cryptorchidism for sons born to mothers with elevated PBDE levels in breast milk (Main et al., 2007). In addition, Chao et al. found that elevated levels of PBDEs in breast milk correlated with decreased infant birth weight, infant birth length, infant chest circumference and infant body mass index (Chao et al., 2007). Elevated levels of PBDEs in human umbilical cord blood have been correlated with preterm birth, low birth weight or stillbirth (Wu et al., 2010). Although these studies report associations between PBDE exposure and adverse birth outcomes, and PBDEs distribute to human placenta (Frederiksen et al., 2009), extraplacental membranes (Miller et al., 2009), amniotic fluid (Miller et al., 2012), and umbilical cord blood (Frederiksen et al., 2009), studies of mechanisms by which PBDEs act on gestational tissues during pregnancy are limited. Specifically, we identified one study reporting that pre-exposure of placental explants to a PBDE mixture of congers 47, 99 and 100 enhanced placental pro-inflammatory response to heat-killed *Escherichia Coli*, with increased PGE2 release and cyclooxygenase (COX)-2 expression (Peltier et al., 2012).

Our previous study showed that treatment with BDE-47, one of the most prevalent congeners found in human tissues (Hites, 2004), stimulates production of the proinflammatory cytokine IL-6 via a reactive oxygen species (ROS)-mediated mechanism in the first trimester EVT human placental cell line HTR-8/SVneo (Park et al., 2014b). Although inappropriate activation of prostaglandin pathways may lead to placental dysfunction, there is a paucity of reports on PBDE-stimulated prostaglandin release in first trimester placenta. Increased oxidative stress in placenta, possibly due to increased generation of ROS, has been observed in pathological pregnancies, and ROS have been implicated in the activation of inflammatory responses in gestational compartments (Buhimschi et al., 2003; Cindrova-Davies et al., 2007). Moreover, formation of ROS has been shown to modulate prostaglandin pathways in various experimental models including murine placenta (Basu, 1999; Davidge, 1998; Wentzel et al., 1999; White et al., 2002).

The present study examines the hypothesis that BDE-47 stimulates PGE2 production in human placental cells via a ROS-mediated mechanism. This work was performed with the HTR-8/SVneo cell line (Graham et al., 1993). The HTR-8/SVneo cell line was derived from first trimester placentae and has provided a useful cell culture model for studies of EVT cellular responses (Liu et al., 2012; Wang et al., 2012; Weber et al., 2013) and initial investigations of toxicant actions on EVTs (Park et al., 2014a; Tetz et al., 2013a).

2. Materials and methods

2.1. Chemicals and assay kits

BDE-47 was purchased from AccuStandard (New Haven, CT, USA). Dimethyl sulfoxide (DMSO), *tert*-butyl hydroperoxide (TBHP), indomethacin, NS398, and (±)-α-tocopherol were purchased from Sigma Aldrich (St. Louis, MO, USA). Purchase of 6-carboxy dichlorodihydrofluorescein diacetate (carboxy-H2DCF-DA), Hoechst 33342 dye, RPMI 1640 medium, fetal bovine serum (FBS), OptiMem 1 reduced-serum medium, Hank's balanced salt solution (HBSS), and 0.25% trypsin/EDTA solution and penicillin/streptomycin (P/S) were from Invitrogen Life Technologies (Carlsbad, CA, USA). The PGE2 ELISA kit and arachidonic acid were purchased from Cayman Chemical (Ann Arbor, MI, USA). QIAshredder, RNeasy mini plus kit, RT^2 First Strand kit for reverse transcriptase reaction, RT^2 qPCR SYBR Green/ROX Master Mix, and primers for human β-microglobulin, PTGS2, PTGES and HPGD were purchased from Qiagen (Valencia, CA, USA). The NP-40 substitute, IGEPAL CA-630, was purchased from United States Biological (Salem, MA). PhosStop protease inhibitor cocktail and complete mini protease inhibitor cocktail tablets were from Roche (Indianapolis, IN). Reducing Laemmli SDS sample buffer was purchased from Boston BioProducts (Ashland, MA). Memcode reversible protein staining kit and bicinchoninic acid (BCA) assay kit were from Thermo Scientific (Waltham, MA). Alkaline phosphatase-linked secondary antibody was purchased from Cell Signaling Technology (Beverly, MA). Enhanced chemifluorescence (ECF) substrate and PVDF membrane Hybond-P were purchased from GE Healthcare Life Sciences (Pittsburgh, PA).

2.2. Cell culture and treatment

The human first trimester extravillous trophoblast cell line HTR-8/SVneo was kindly provided by Dr. Charles S. Graham (Queen's University, Kingston, ON, Canada). Cytotrophoblast cells isolated from first trimester human placentae were immortalized with simian virus 40 large T antigen to generate the HTR-8/SVneo cell line (Graham et al., 1993). Cells between passages 71 and 84 were cultured in RPMI 1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin at 37 °C in a 5% CO_2 humidified atmosphere. Cells were grown to 70–90% confluence before treatment. Cells were washed twice with OptiMem 1 containing 1% FBS and 1% P/S, and then acclimated with the medium for 1 h at 37 °C. From solutions of 5, 10, 15 and 20 mM BDE-47 in DMSO, exposure media containing 5, 10, 15 and 20 μM BDE-47 were made in OptiMem 1 containing 1% FBS and 1% P/S immediately prior to initiating the experiments. BDE-47 concentrations were selected to include concentrations relevant to human exposure (Doucet et al., 2009) and previously shown by us to increase generation of ROS in the HTR-8/SVneo cells (Park et al., 2014b). The final concentration of DMSO in medium was 0.7% (v/v).

2.3. Carboxydichlorofluorescein assay

Stimulation of ROS generation was assessed using carboxydichlorofluorescein (cDCF) fluorescence in a variation of the dichlorofluorescein (DCF) assay. We used cDCF instead of DCF because the additional negative charges on cDCF improve cell retention of the probe. Because artifactual results can occur in the cDCF assay

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