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Trichloroethylene metabolite S-(1,2-dichlorovinyl)-L-cysteine induces lipid peroxidation-associated apoptosis via the intrinsic and extrinsic apoptosis pathways in a first-trimester placental cell line

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ABSTRACT

Trichloroethylene (TCE), a prevalent environmental contaminant, is a potent renal and hepatic toxicant through metabolites such as S-(1, 2-dichlorovinyl)-L-cysteine (DCVC). However, effects of TCE on other target organs such as the placenta have been minimally explored. Because elevated apoptosis and lipid peroxidation in placenta have been observed in pregnancy morbidities involving poor placentation, we evaluated the effects of DCVC exposure on apoptosis and lipid peroxidation in a human extravillous trophoblast cell line, HTR-8/SVneo. We exposed the cells in vitro to 10–100 μM DCVC for various time points up to 24 h. Following exposure, we measured apoptosis using flow cytometry, caspase activity using luminescence assays, gene expression using qRT-PCR, and lipid peroxidation using a malondialdehyde quantification assay. DCVC significantly increased apoptosis in time- and concentration-dependent manners ($p < 0.05$). DCVC also significantly stimulated caspase 3, 7, 8 and 9 activities after 12 h ($p < 0.05$), suggesting that DCVC stimulates the activation of both the intrinsic and extrinsic apoptotic signaling pathways simultaneously. Pre-treatment with the tBID inhibitor Bl-6C9 partially reduced DCVC-stimulated caspase 3 and 7 activity, signifying crosstalk between the two pathways. Additionally, DCVC treatment increased lipid peroxidation in a concentration-dependent manner. Co-treatment with the antioxidant peroxyl radical scavenger (±)-α-tocopherol attenuated caspase 3 and 7 activity, suggesting that lipid peroxidation mediates DCVC-induced apoptosis in extravillous trophoblasts. Our findings suggest that DCVC-induced apoptosis and lipid peroxidation in extravillous trophoblasts could contribute to poor placentation if similar effects occur in vivo in response to TCE exposure, indicating that further studies into this mechanism are warranted.

1. Introduction

Trichloroethylene (TCE) is a chlorinated volatile organic solvent most commonly used in chemical production and as an industrial metal degreaser (Waters et al., 1977; Chiu et al., 2013; NTP, 2015). Ranked as number sixteen on the U.S. Agency for Toxic Substances and Disease Registry's Priority List of Hazardous Substances, TCE is a common environmental contaminant found in approximately 800 Environmental Protection Agency-designated Superfund sites (Chiu et al., 2013; ATSDR, 2015). Despite being classified as a "known human carcinogen," (Guha et al., 2012; NTP, 2015), approximately 1.9 million pounds of TCE were released into the environment in 2015 (EPA, 2017). Because of its continued use and widespread persistent environmental contamination, TCE exposure continues to pose a threat to human health through ingestion of contaminated drinking water and inhalation of the volatilized chemical.

Although TCE is most commonly recognized as a renal and liver toxicant (Chiu et al., 2013), its effects during pregnancy are not well

Abbreviations: BAK1, BCL2-antagonist/killer 1; BAX, BCL2-associated X protein; BCL-2, B-cell CLL/lymphoma 2; BCL2A1, BCL2-related protein A1; BID, BH3 interacting domain death agonist; CASP1, Caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase); CASP3, Caspase 3, apoptosis-related cysteine peptidase; CASP4, Caspase 4, apoptosis-related cysteine peptidase; CASP7, Caspase 7, apoptosis-related cysteine peptidase; CASP8, Caspase 8, apoptosis-related cysteine peptidase; CASP9, Caspase 9, apoptosis-related cysteine peptidase; DCVC, S-(1, 2-dichlorovinyl)-L-cysteine; DEVD, Asp-Glu-Val-Asp; DFFA, DNA fragmentation factor, 45 kDa, alpha polypeptide; FAS, Fas (TNF receptor superfamily, member 6); FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; GADD45A, Growth arrest and DNA-damage-inducible, alpha; HRK, Harakiri, BCL2 interacting protein (contains only BH3 domain); LEHD, Leu-Glu-His-Asp; LETD, Leu-Glu-Thr-Asp; IGF1R, Insulin-like growth factor 1 receptor; MCL, maximum contaminant level; MDA, malondialdehyde; NFKB1, Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1; RIPK2, Receptor-interacting serine-threonine kinase 2; ROS, reactive oxygen species; TBA, thiobarbituric; TBHP, *tert*-butyl hydroperoxide; tBID, truncated BH3 interacting domain death agonist; TCE, trichloroethylene; TP53, tumor protein p53; TRAF3, TNF receptor-associated factor 3

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understood. TCE-induced fetal cardiotoxicity is particularly controversial: for example, some laboratory animal studies reported TCE effects (Das and Scott, 1994; Johnson et al., 2003; Rufer et al., 2010) and other studies reported no cardiac defects (Fisher et al., 2001; Carney et al., 2006). Furthermore, two systematic literature reviews concluded there was insufficient evidence to support an association between TCE exposure and congenital heart abnormalities (Watson et al., 2006; Bukowski, 2014). With regard to other pregnancy outcomes, an earlier study found no association between maternal TCE exposure and low birth weight (Lagakos et al., 1986), but more recent epidemiology studies report positive associations of TCE exposure during pregnancy with decreased fetal weight and preterm birth (Forand et al., 2012) (Ruckart et al., 2014).

Placental toxicity could potentially mediate adverse birth outcomes such as preterm birth and decreased birth weight (Ilekis et al., 2016). In particular, emerging recent studies implicate placental insufficiency, defined as inadequate maternal-fetal nutrient and waste exchange resulting from placental abnormalities, as a potential cause of premature labor (Morgan, 2014; Morgan, 2016) Furthermore, a recent epidemiology study found a significant association between pre-eclampsia and preterm birth (Davies et al., 2016). Because it is highly perfused, the placenta is readily exposed to circulating TCE and its metabolites, and may be a target for TCE toxicity (Laham, 1970). Moreover, the placenta is capable of metabolizing compounds, which puts it at risk for tissue generation of toxic TCE metabolites (Burton and Fowden, 2015).

Previous studies have shown that TCE exerts its toxic effects primarily through its metabolites (Lash et al., 2014). For example, exposure to the glutathione conjugation pathway metabolite S-(1, 2-dichlorovinyl)-L-cysteine (DCVC) is toxic in vitro to renal proximal tubular cells, the main putative target in the kidney, of rats, mice and humans (Lash and Anders, 1986; Darnerud et al., 1989; Chen et al., 2001; Lash et al., 2001; Xu et al., 2008). Furthermore, numerous studies demonstrated that DCVC induces mitochondrial dysfunction, excessive reactive oxygen species generation and subsequent lipid peroxidation in kidney cells (Lash and Anders, 1986; Chen et al., 1990; van de Water et al., 1994; van de Water et al., 1995; Chen et al., 2001; Lash et al., 2003; Xu et al., 2008). Similarly, our lab recently showed that DCVC induces a loss of mitochondrial membrane potential and increases ROS generation in the first trimester extravillous trophoblast HTR-8/SVneo cell line (Hassan et al., 2016). Taken together, the evidence indicates that mitochondrial dysfunction and aberrant ROS-generating lipid peroxidation play central roles in DCVC-mediated TCE cytotoxicity (Lash and Anders, 1986).

Mitochondria and lipid peroxidation are involved in the regulation of apoptotic pathways (Simon et al., 2000; Ayala et al., 2014), and multiple studies have demonstrated that DCVC initiates apoptosis in proximal tubular cells of humans and rodents (Van de Water et al., 1996; Chen et al., 2001; Lash et al., 2001; Xu et al., 2008). Apoptosis is a highly organized form of cell death that is tightly regulated by two primary signaling pathways depending upon the stimulus: the mitochondrial-dependent or intrinsic pathway and the cell surface death receptor-mediated or extrinsic pathway. Apoptosis is especially critical during pregnancy (Straszewski-Chavez et al., 2005). During placental development, apoptosis plays an important role in removing damaged cells without injuring surrounding tissues (Smith et al., 1997b; Straszewski-Chavez et al., 2005; Sharp et al., 2010). Despite its role in normal placentation, evidence suggests that an abnormal increase in apoptosis of extravillous trophoblasts contributes to multiple placental pathologies including intrauterine growth restriction and pre-eclampsia (Smith et al., 1997a; DiFederico et al., 1999; Genbacev et al., 1999). The present study investigated the effects of DCVC on the two primary apoptosis signaling pathways and lipid peroxidation in the human extravillous trophoblast cell line HTR-8/SVneo.

2. Materials and methods

2.1. Chemicals and reagents

The trichloroethylene metabolite S-(1, 2-dichlorovinyl)-L-cysteine (DCVC) was synthesized by the University of Michigan Medicinal Chemistry Core according to procedures described by McKinney et al. (McKinney et al., 1959). Purity (98.7%) was determined by HPLC analysis. A stock solution was prepared in PBS and identity was confirmed by proton nuclear magnetic resonance spectroscopy performed at the University of Michigan Biochemical Nuclear Magnetic Resonance Core. Phosphate buffered saline (PBS) and 0.25% trypsin were purchased from Invitrogen Life Technologies (Carlsbad, CA, USA). BI-6C9 tBID inhibitor, *tert*-butyl hydroperoxide (TBHP) and (±)-α-tocopherol were purchased from Sigma-Aldrich (St. Louis, MO). Camptothecin was purchased from Cayman Chemical (Ann Arbor, MI). RPMI 1640 culture medium with L-glutamine and without phenol red, 10,000 U/mL penicillin/10,000 μg/mL streptomycin (P/S) solution, and fetal bovine serum (FBS) were purchased from Thermo Fisher Scientific (Waltham, MA, USA).

2.2. Cell culture and treatment

The HTR-8/SVneo cell line, a gift from Dr. Charles H. Graham (Queen's University, Kingston, Ontario, Canada), models first-trimester extravillous cytotrophoblasts in vitro (Graham et al., 1993). HTR-8/SVneo cells were originally derived from first trimester female human cytotrophoblast cells and immortalized with simian virus 40 large T antigen (Graham et al., 1993). HTR-8/SVneo cells were cultured as previously described (Tetz et al., 2013; Hassan et al., 2016). Briefly, cells were cultured between passages 71–87 in RPMI 1640 medium supplemented with 10% FBS and 1% P/S at 37 °C in a 5% CO_2 humidified incubator. Cells were maintained in RPMI 1640 growth medium with 10% FBS and 1% P/S prior to and during experiments to ensure optimal cell growth (Graham et al., 1993). Cells were grown to 70–90% confluence at least 24 h after subculture before starting any experiment. A stock solution of 1 mM DCVC was prepared in PBS and stored in 1 mL aliquots at − 20 °C to minimize freeze/thaw cycles. Prior to each experiment, a DCVC stock solution aliquot was quickly thawed in a 37 °C water bath and then diluted in RPMI 1640 medium with 10% FBS and 1% P/S to final exposure concentrations of 10–100 μM DCVC. The DCVC concentrations were selected for the current study to include the mean peak blood concentration of 13.4 μM *S*-(1,2-dichlorovinyl)glutathione, the stable precursor of DCVC, measured in female volunteers exposed to 100 ppm of TCE by inhalation for 4 h (Lash et al., 1999), with higher concentrations consistent with DCVC-induced cytotoxicity in human placental cells and human proximal tubular cells in vitro (Xu et al., 2008; Hassan et al., 2016).

2.3. Cell line validation

HTR-8/SVneo cells were seeded at a density of 400,000 cells per well in a 6-well cell culture plate and allowed to adhere for 24 h. Cells were treated with RPMI 1640 medium alone for 24 h. Following exposure, DNA was extracted using QIAamp® DNA Mini Kit (Qiagen; Hilden, Germany). RNA samples were frozen at − 20 °C and transported to the University of Michigan DNA Sequencing Core for completion of the cell line validation process using microsatellite genotyping. At the core, AmpFLSTR Identifiler Plus PCR Amplification Kit run on an 3730XL Genetic Analyzer purchased from Applied Biosystems (Waltham, MA) was utilized to identify human genomic DNA for 8 tetranucleotide repeat loci and the Amelogenin gender determination marker. The short tandem repeat profile generated for our cells was compared to the short tandem repeat profile for HTR-8/SVneo (ATCC® CRL-3271™) published by American Type Culture Collection (Manassas, VA) (ATCC, 2015). The short tandem repeat profile was an exact match: