Study of the effects of bisphenol A using human fetal lung fibroblasts

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Bisphenol A (BPA, 2,2-bis(4-hydroxyphenyl) propane; CAS# 80-05-7) is a highly produced industrial chemical, with an estimated four billion kilograms produced worldwide each year (reviewed in Vandenberg et al., 2010). BPA serves as a monomer in the manufacture of polycarbonate, a hard, clear plastic. Polycarbonate plastics are used in many consumer products, such as reusable water bottles. BPA is also widely used to make epoxy resins, which are used as coating on the inside of some metal-based food and beverage cans to protect the food from direct contact with metal surfaces. BPA has been used in food packaging since the 1960s. Small amounts of BPA can be leached out from the containers into food and drinks. Because of the prevalence of BPA in food and beverage containers, humans are exposed to measurable levels of BPA (Krishnan et al., 1993; Staples et al., 1998; Kang et al., 2006; Calafat et al., 2008; Vandenberg et al., 2010; Wang et al., 2014). For example, the general population in the United States consumes a median BPA amount of 34 nanograms per kilogram body weight per day (34 ng/kg day) (Lakind and Naiman, 2011). The major route of human exposure to BPA is through ingestion, although dermal exposure to BPA is also possible (Liao and Kannan, 2011), because newspapers, flyers, tickets, receipts (thermal paper), and magazines also contain BPA.

Many studies have focused on the effects of BPA, including its possible links to diabetes, obesity, reproductive disorders, cardiovascular diseases, birth defects, kidney diseases, and cancer (Wetherill et al., 2007; Rezg et al., 2014; Li et al., 2015; Yin et al., 2015; Seachrist et al., 2016). BPA is known for its endocrine-disrupting estrogenic effects, which have been referenced to contribute to chronic diseases (Wetherill et al., 2007; Rubin, 2011; Rezg et al., 2014). Canada, the European Union, the United States, and several countries in Asia have banned the use of BPA for the production of baby bottles ( EUR-Lex, 2011; Government of Canada, 2013; USFDA, 2014; Baluka and Rumbeiha, 2016). However, conflicting findings on BPA toxicity and the reported differences between low-dose and high-dose effects have led to continuing debate over the effects of BPA and its mechanism(s) of action (Vandenberg et al., 2009; Valentino et al., 2016). As a result, several legislations have deemed BPA irrelevant to human health and still permit the wide use of BPA for the production of select materials.

An active topic of research focuses on the role of BPA in the development of asthma (Robinson and Miller, 2015). Most experiments on this topic have been performed on mice pups, and these experiments demonstrated the adverse effects of BPA on normal fetal lung and airway development. Fetal lung impairments resulted from high concentrations of BPA in maternal diet. Effects included diminished alveolar airspace, increased alveolar septa thickness and immature alveolar epithelial cells (Hiizumi et al., 2015). In addition, exposure to high concentrations of BPA in utero alters prenatal development of the murine immune system, leading to allergic sensitization and eosinophil recruitment that result in asthma phenotypes after birth (Nakajima et al., 2012; Midoro-Horiuti et al., 2010). However, other studies suggest that exposure to BPA during fetal stages of lung development does not cause any significant allergic lung inflammation observable in adulthood (Bauer...
Even with studies that demonstrate clear linkage between BPA and asthma, it is difficult to reliably translate the results from high-dose exposure in animal models to low-dose scenarios in human populations.

Epidemiological studies of human populations suggest that BPA contributes to the prenatal and postnatal development of asthma. On average, the concentration of BPA in the placenta during pregnancy ranges from 1.0 to 104.9 ng/g and the average fetal plasma concentrations ranges from 0.2 to 9.2 ng/mL (Schonfelder et al., 2002). Prenatal exposure to BPA has been correlated with diminished lung function in young children less than 5 years old (Spanier et al., 2014). Urinary BPA concentrations in early childhood were also correlated with asthma development later in childhood in a cohort study (Donohue et al., 2013). However, the same study suggests that prenatal BPA exposure did not correlate with the prevalence of asthma in the children. Thus, there appears to be conflicting results between pre/postnatal BPA exposure and childhood development of asthma, which could be due to the variations in the populations studied.

Attempting to gain further insights into the potential role of BPA in possible asthma development, Mahemuti et al. (2016) tested the effects of BPA on developmental and immune responses in human fetal lung fibroblasts (hFLFs) (Fig. 1). Performing such an in vitro experiment complements animal and epidemiological studies by elucidating direct cellular responses of human lung cell-lines to BPA that may underlie asthma phenotypes. Studying fibroblasts separated from in vivo conditions is appealing because these fibroblasts respond to and also produce inflammatory mediators (Alkhouri et al., 2014), and they secrete extracellular matrix for tissue remodeling that may become aberrant in lung diseases, including asthma (Fixman et al., 2007; Dijkstra et al., 2006). Previously, in vitro experiments have been beneficial when illustrating causality between BPA and chronic diseases. For example, epidemiological findings that correlate BPA and type-2 diabetes have been further supported by in vitro findings that show that physiologically relevant concentrations of BPA can alter immune factor expression and insulin signaling in 3 T3-L1 pre-adipocytes (Ahmadkhaninia et al., 2014; Valentino et al., 2013). Studying the effects of BPA on hFLFs may help confirm the presence or absence of asthmogenic cellular pathways underlying the correlations between BPA and asthma identified in epidemiological and animal studies.

Because BPA is an estrogen analog capable of interrupting signaling pathways of estrogen receptors (ERs), it is plausible that BPA may have similar effects to those of estrogens. Estrogen receptor alpha (ERα) and beta (ERβ) are expressed in the lung in response to exposure to estrogens (Mollerup et al., 2002; Ciana et al., 2001; Marino et al., 2006). Estrogens also affect pulmonary alveolar development (Massaro and Massaro, 2004; Massaro et al., 2007). Lung pathology often has signs of altered ER expression (Logginidou et al., 2000). In addition, BPA may also act through the membrane receptor G protein-coupled receptor 30 (GPR30) (Prossnitz et al., 2007) and affect downstream messengers in the lung, which has been implicated in other lung pathologies (Zhang et al., 2014). As a result, Mahemuti et al. (2016) sought to detect the presence of estrogen receptors ERα, ERβ, and GPR30 in hFLFs. Using fluorescence microscopy, they found that all three ERs were stained in the nuclei and cytoplasm, with a denser localization within the nuclei. The presence of ERs in the nuclei and cytoplasm of hFLFs suggests the potential for BPA to disrupt airway development and function through ER signaling (Bonds and Midoro-Horiuti, 2013).

Maternal and fetal BPA concentrations in the serum and placenta are well-documented (Schonfelder et al., 2002), but the amount of BPA absorbed into lung cells, specifically hFLFs, was not known. Mahemuti et al. (2016) used gas chromatography separation with mass spectrometry detection to measure the intracellular concentration of BPA. They detected the highest intracellular BPA levels to be 23 μmol/L in hFLFs incubated with 100 μmol/L BPA. However, they found that the ratio of intracellular to extracellular BPA concentrations decreased with increasing BPA administration. They suggest that either the BPA binding sites in hFLFs could have been saturated or that BPA excretion could have equilibrated with BPA absorption at the higher levels of exposure to BPA.

BPA appears to cause mitochondria-mediated apoptosis and necrosis in other tissue cells (Xia et al., 2014; Lin et al., 2013; Neri et al., 2015), but it is unknown if BPA induces similar effects in lung cells. Mahemuti et al. (2016) show that hFLFs seemed to survive even 100 μmol/L BPA in the culture medium. However, the hFLFs had a fibrotic appearance, which is indicative of cell stress and injury (Cheresh et al., 2013). Therefore, it is likely that high doses of BPA stressed the hFLFs enough to activate an adaptive response. This coincides with their finding of increased growth differentiation factor 15 (GDF15), which could be responsible for the fibrotic appearance (Lambrecht et al., 2014), and stress resistance (Nickel et al., 2011;
proteinases (Royce et al., 2012; Linthout et al., 2011), inflammation, such as macrophage recruitment, matrix metalloproteinases (Royce et al., 2012; Linthout et al., 2011), interleukins, and lymphocytes (Amin et al., 2000), may provide useful insights. These factors could play a role in fetal airway structural changes and asthma development before birth. The presence of apoptotic and necrotic factors, such as caspases and cytosolic adenylate kinase, could be evaluated to understand the cytotoxicity of BPA. Lastly, applying the in vitro approach of studying direct cellular responses to BPA to already differentiated lung cells, such as endothelial, smooth muscle, and alveolar cells, would help illustrate impairments to fetal lung tissue by BPA.

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