JOURNAL OF ENVIRONMENTAL SCIENCES 48 (2016) 11–23

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jes

JES JOURNAL OF ENVIRONMENTAL SCIENCES

www.jesc.ac.cn

Bisphenol A exposure alters release of immune and developmental modulators and expression of estrogen receptors in human fetal lung fibroblasts

Laziyan Mahemuti[1,5], *Qixuan Chen*[1], *Melanie C. Coughlan*[1], *Min Zhang*[1], *Maria Florian*[1,5], *Ryan J. Mailloux*[1,5], *Xu-Liang Cao*[2], *Kylie A. Scoggan*[3,4], *William G. Willmore*[5], *Xiaolei Jin*[1,*]

1. *Regulatory Toxicology Research Division, Bureau of Chemical Safety, Food Directorate, HPFB, Health Canada, Ottawa, Ontario, Canada. E-mail: Mahemuti_Laziyan@hc-sc.gc.ca*
2. *Food Research Division, Bureau of Chemical Safety, Food Directorate, HPFB, Health Canada, Ontario, Canada*
3. *Nutrition Research Division, Bureau of Nutritional Sciences, Food Directorate, HPFB, Health Canada, Ottawa, Ontario, Canada*
4. *Sector Strategies Division, Risk Management Bureau, Safe Environments Directorate, HECSB, Health Canada, Ottawa, Ontario, Canada*
5. *Institute of Biochemistry, Departments of Biology and Chemistry, Carleton University, Ottawa, Ontario, Canada*

ARTICLE INFO

Article history:
Received 8 December 2014
Revised 3 February 2016
Accepted 4 February 2016
Available online 29 April 2016

Keywords:
Bisphenol A
Human fetal lung fibroblasts
Estrogen receptors
Antagonists
Cytokines
Chemokines
Immune and developmental modulators

ABSTRACT

Bisphenol A (BPA) has been shown to exert biological effects through estrogen receptor (ER)-dependent and ER-independent mechanisms. Recent studies suggest that prenatal exposure to BPA may increase the risk of childhood asthma. To investigate the underlying mechanisms in the actions of BPA, human fetal lung fibroblasts (hFLFs) were exposed to varying doses of BPA in culture for 24 hr. Effects of BPA on localization and uptake of BPA, cell viability, release of immune and developmental modulators, cellular localization and expression of ERα, ERβ and G-protein coupled estrogen receptor 30 (GPR30), and effects of ERs antagonists on BPA-induced changes in endothelin-1 (ET-1) release were examined. BPA at 0.01–100 μmol/L caused no changes in cell viability after 24 hr of exposure. hFLFs expresses all three ERs. BPA had no effects on either cellular distribution or protein expression of ERα, however, at 100 μmol/L (or 23 μmol/L intracellular BPA) increased ERβ protein levels in the cytoplasmic fractions and GPR30 protein levels in the nuclear fractions. These paralleled with increased release of growth differentiation factor-15, decreased phosphorylation of nuclear factor kappa B p65 at serine 536, and decreased release of ET-1, interleukin-6, and interferon gamma-induced protein 10. ERs antagonists had no effects on BPA-induced decrease in ET-1 release. These data suggest that BPA at 100 μmol/L altered the release of immune and developmental modulators in hFLFs, which may negatively influence fetal lung development, maturation, and susceptibility to environmental stressors, although the role of BPA in childhood asthma remains to be confirmed in *in vivo* studies.

* *Corresponding author*. E-mail: Dawn_Jin@hc-sc.gc.ca; jxnc329@gmail.com (Xiaolei Jin).

http://dx.doi.org/10.1016/j.jes.2016.02.013
1001-0742/

Introduction

The 2,2-Bis (4-hydroxyphenyl) propane, more commonly known as bisphenol A (BPA), is a chemical monomer used primarily in the production of polycarbonate plastics and epoxy resins. Polycarbonate has been used in food contact materials such as beverage bottles, infant feeding bottles, and food containers. Epoxy resins are used in protective linings of cans containing foods and beverages, as well as infant formula products. This has raised concern in the scientific community over the possible developmental effects of BPA in humans. It is known that BPA is rapidly transferred to the fetus after maternal uptake (Takahashi and Oishi, 2000), and that repeated maternal exposure elevates BPA in fetus or the newborn (Durando et al., 2007). However, the levels of fetal exposure to BPA in general population remain to be established. Available information on placenta, fetal blood, amniotic fluid, and fetal liver BPA concentrations suggests a wide range of distribution from undetectable to hundreds of ng/g (Vandenberg et al., 2012; Cao et al., 2012; Edlow et al., 2012; Ikezuki et al., 2002). The human fetal lung is exposed to BPA through both blood and amniotic fluid, however, has not been assessed for BPA levels. With limited detoxification capacity and high surface to volume ratio, fetal lung cells may be exposed to many folds higher BPA concentrations than cells of other fetal tissues.

Exposure to BPA has been linked to a number of developmental and reproductive pathologies in both animal models and humans (Golub et al., 2010; Palanza et al., 2008; Tharp et al., 2012). However, the underlying molecular mechanisms remain to be elucidated. BPA is traditionally considered as an estrogenic endocrine disrupter with some effects being estrogen receptor (ER)-dependent (Yoshitake et al., 2008; Buteau-Lozano et al., 2008; Dang et al., 2007). However, some recent studies demonstrated that it can also act through ER-independent mechanisms (Hanet et al., 2008; Asahi et al., 2010). Estrogens and ERs are known to enhance allergic sensitization in animal models and may enhance susceptibility to atopic disorders like asthma in humans (Bonds and Midoro-Horiuti, 2013). ERα polymorphisms are associated with airway hyper-responsiveness and lung function decline, particularly in female subjects with asthma (Dijkstra et al., 2006). Not only do endogenous estrogens play a role in the pathogenesis of lung diseases, but environmental estrogens (xenoestrogens) can also have similar effects. BPA was found to enhance allergic sensitization and bronchial inflammation and responsiveness in a susceptible animal model of asthma (Midoro-Horiuti et al., 2010). Prenatal exposure to BPA was associated with increased odds of wheeze in children at 6 months of age (Spanier et al., 2012), increased risk of developing experimental allergic asthma in mice (Nakajima et al., 2012), and enhanced airway lymphocytic and lung inflammation in mucosal sensitized female offspring of mice (Bauer et al., 2012). Hijazi et al. (2015) demonstrated that prenatal exposure to BPA caused lung immaturity phenotype in mice, which was rescued by maternal administration of dexamethasone, an inducer of glucocorticoid. While these studies suggest that fetal exposure to BPA may delay lung maturation and enhance susceptibility to respiratory sensitization and responsiveness, the underlying mechanisms of such effects and the role of ERs remain to be elucidated.

Abnormalities of extracellular matrix (ECM) are a key feature of tissue remodeling in lung disease (Fixman et al., 2007). Lung fibroblasts are known to release many types of immune and developmental modulators including cytokines and chemokines in the ECM (Alkhouri et al., 2014). Thus, it is plausible to hypothesize that exposure of the fetal lung fibroblasts to BPA alters the release of cytokines, chemokines and/or costimulatory molecules, affecting the development, maturation, and susceptibility of fetal lung to environmental stressors, contributing to increased risk of childhood asthma. In this study, therefore, we examined the protein expression and localization of ERs and the effects of BPA on the release of growth differentiation factor-15 (GDF15), ET-1, interleukin-6 (IL-6), and interferon gamma induced protein 10 (IP-10), and phosphorylation of nuclear factor kappa B p65 (NF-κB p65) in cultured normal human fetal lung fibroblasts (hFLFs) and the potential role of ERs in the action of BPA in this cell line.

1. Materials and methods

1.1. Chemicals and doses

BPA powder (99%) was purchased from Sigma-Aldrich (Oakville, Ontario, Canada). A 1 mmol/L stock solution of BPA was prepared and diluted in distilled and deionized water to obtain 0.1, 1, 10, 100 and 1000 μmol/L BPA as working stock solutions. We used 0, 0.01, 0.1, 1, 10, 20, 50 and 100 μmol/L BPA doses in our experiments. These BPA doses were chosen because in a previous study, we found that experimental materials, buffer, serum and media are all contaminated with BPA at concentrations ranging from 0.088 to 19 nmol/L (Cao et al., 2010). The Eagle's Minimal Essential Medium (EMEM) with serum contains 1.93 nmol/L of BPA, which reflects the BPA concentration in our control culture. Any concentrations lower than 1.93 nmol/L is not achievable under the conditions used.

1.2. Cell culture

Normal human fetal lung fibroblasts (hFLFs) (WI-38, CCL-75) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cell culture medium and fetal calf serum were obtained from Invitrogen-Gibco (Carlsbad, CA, USA). Cells were maintained in phenol red-free EMEM with 10% fetal bovine serum at 37°C and 5% CO_2.

1.3. Cellular BPA content and localization

The hFLFs (4×10^4 cells/mL or 4×10^5 cells/dish) were seeded in 10 cm culture dishes overnight, then treated with 0, 0.01, 0.1, 1, 10, 20, 50, or 100 μmol/L BPA for 24 hr. Cells were harvested in a graduated micro glass tube for volume measurement, and then lysed in Pierce RIPA buffer (Thermo Scientific, Rockford, IL, USA). Cell lysates were extracted with acetonitrile followed by further extraction and clean-up using Bond Elut C18 solid phase extraction (SPE) cartridges (500 mg, 6 ml capacity) from Agilent (Mississauga, ON, Canada). The