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Bisphenol A, an environmental estrogen-like toxic chemical, induces cardiac fibrosis by activating the ERK1/2 pathway

Yingying Hu[a,b,1], Li Zhang[c,1], Xianxian Wu[a,1], Liangyu Hou[a], Zhange Li[a], Jin Ju[a], Qian Li[d], Wei Qin[a], Jiamin Li[a], Qingwei Zhang[a], Tong Zhou[a], Longyin Zhang[a], Chaoqian Xu[a], Zhiwei Fang[a], Yong Zhang[a,e,f,*]

[a] Department of Pharmacology, State-Province Key Laboratories of Biomedicine-Pharmaceutics of China, Key Laboratory of Cardiovascular Research, Ministry of Education, Harbin Medical University, Harbin 150081, China
[b] Department of Pharmacy, The First Affiliated Hospital of Harbin Medical University, Harbin 150001, China
[c] Institute of Clinical Pharmacology, The Second Affiliated Hospital, Key Laboratory of Drug Research, Heilongjiang Higher Education Institutions, Harbin Medical University, Harbin 150086, China
[d] Department of Pharmaceutical Analysis, College of Pharmacy, Harbin Medical University, Harbin 150081, China
[e] Institute of Metabolic Disease, Heilongjiang Academy of Medical Science, Harbin 150086, China
[f] Wu Lien–Teh Institute, Harbin Medical University, Harbin 150086, China

HIGHLIGHTS

- Exposure to a high dosage of bisphenol A (BPA) comparable to its urinary concentration decreased cardiac function.
- BPA induced cardiac fibrosis.
- BPA markedly facilitated proliferation and collagen production of cardiac fibroblasts by activating ERK1/2.
- Antiestrogen or ERK inhibitor prevented BPA-induced proliferation and collagen production of cardiac fibroblasts, indicating that BPA acts by activating estrogen receptor and the ERK1/2-dependent pathways.

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ABSTRACT

Bisphenol A (BPA) is a widely studied typical endocrine-disrupting chemical. The present study aimed to verify whether BPA could induce proliferation of cardiac fibroblasts and collagen production leading to cardiac interstitial fibrosis. After exposure to BPA for 30 consecutive days, decreased cardiac function was observed in rats using echocardiography, and the deposition of collagen was detected by Masson's trichrome staining and electron microscope. BPA remarkably stimulated proliferation and migration of cultured cardiac fibroblasts and collagen production in a concentration-dependent manner, as revealed by MTT, wound healing assay and collagen assay. Meanwhile, BPA treatment also enhanced phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2). In contrast, pretreatment with estrogen receptor inhibitor ICI182780 or ERK inhibitor PD98059 prevented the enhanced phosphorylation of ERK1/2, and subsequently inhibited the up-regulation of transforming growth factor-β1 (TGF-β1) expression induced by BPA. As a consequence, these inhibitors also decreased proliferation and collagen production, as well as the fibrosis-related genes expression. Taken together, our results indicated that BPA may act as a promoting factor in proliferative process and collagen production of cardiac fibroblasts via activating ERK1/2.

Abbreviations: BPA, bisphenol A; EF, ejection fraction; ECM, extracellular matrix; ER, estrogen receptor; ERK1/2, extracellular signal-regulated kinase 1/2; FS, fractional shortening; GPER, G protein-coupled receptor; IVS, interventricular septum; JNK, c-Jun *N*-terminal kinase; LVID, left ventricular internal dimension; LVPW, left ventricular posterior wall; MAPK, mitogen-activated protein kinase; TGF-β1, transforming growth factor-β1.

* Corresponding author.
E-mail addresses: hmuzhangyong@hotmail.com, zy@ems.hrbmu.edu.cn (Y. Zhang).

[1] These authors contributed equally to this study.

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1. Introduction

Bisphenol A (BPA) has been frequently used in a wide range of consumer products, including toys, drinking bottles, food containers and dental sealants, and become ubiquitous in the environment (Fenichel et al., 2013). As one of the environmental estrogenic endocrine disruptors, BPA possesses similar chemical structure to estradiol and diethylstilbestrol (Fig. 1). Up to 92.6% of the human population has detectable BPA levels in their bodies and there is an increasing concern about its higher bioaccumulation in developing organisms (Calafat et al., 2008; Kitraki et al., 2015). The current standard for tolerable daily intake value of BPA (0.05 mg/kg body weight (bw)/day) was set by the European Food Safety Authority in 2006 (EFSA). However, the reference dose, previously determined as the safe daily human exposure, has recently been reduced to 4 μg/kg/day (http://www.efsa.europa.eu/en/topics/topic/bisphenol.htm) due to increasing evidence for adverse effects at lower exposures.

For the general population with environmentally-relevant exposure, it seems that BPA does not pose threats to human health. But for the people who are under specific conditions or under the occupational exposure, BPA can produce detrimental effects on the body. Patel et al. found that lifelong exposure to BPA (5 μg/kg/day) alters cardiac structure and function in C57BL/6n mice in a sex-specific manner (Patel et al., 2013). Kim et al. showed that chronic exposure to BPA (50 μg/kg/day) for 12 weeks accelerates atherosclerosis progress in ApoE−/− mice (Kim et al., 2014). Meanwhile, more and more studies have provided evidences for the adverse effects of BPA (especially at the high dosages) in different systems both in vivo and in vitro (Ahbab et al., 2015; Fang et al., 2015; Khan et al., 2015; Pan et al., 2015). It has been reported that daily oral administration of BPA (25 mg/kg/day for 6 weeks and 10 mg/kg for 6 and 10 weeks) has effects on oxidative stress parameters, glutathione level and catalase activity in the heart of adult rats (Aboul Ezz et al., 2015). These high-dosage studies may not appropriately reflect the environmentally relevant exposure level of BPA; however, these "high-dosage" studies are of pharmacological interests and may help us to understand the potential targets and signaling pathways involved in its cardiovascular toxicity.

Findings from epidemiological studies have demonstrated that higher BPA urine concentrations are associated with an increased risk of coronary artery disease, hypertension, carotid atherosclerosis, angina and myocardial infarction (Bae et al., 2012; Lang et al., 2008; Lind and Lind, 2011; Melzer et al., 2010; Shankar et al., 2012). Cardiac fibroblasts, the most abundant cell type in the heart, play an important role in providing structural support to the heart and maintaining normal myocardial function by producing extracellular matrix (ECM) such as collagens and fibronectin (Banerjee et al., 2007; Brown et al., 2005; Takeda et al., 2010). However, hyperactivity of cardiac fibroblasts may result in excessive production and deposition of ECM in the myocardium, known as fibrosis. Fibrosis has adverse effects on cardiac structure and function, and is a pathological feature common to numerous forms of heart disease, including myocardial infarction, hypertension, and heart failure (Fan et al., 2012; Shi et al., 2011; Souders et al., 2009).

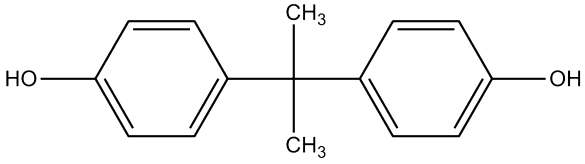

Fig. 1. Chemical structure of Bisphenol A (BPA).

The intracellular mitogen-activated protein kinase (MAPK) signaling cascades regulate diverse cellular programs, including proliferation, differentiation and development, which are known to play an important role in the pathogenesis of cardiac fibrosis (Muslin, 2008). The best known are the conventional MAPKs, including the extracellular signal-regulated kinases 1 and 2 (ERK1/2), p38 kinase and c-Jun N-terminal kinase (JNK). ERK1/2 MAPKs are mainly involved in cell growth and differentiation (Pan et al., 2011; Thum et al., 2008). Previous studies have shown that BPA can induce proliferation of breast cancer cells by activating the MAPK pathway (Pupo et al., 2012; Wu et al., 2012). And previous investigations reviewed by Vandenberg et al. have demonstrated that BPA can bind to and activate the estrogen receptor (ER-α and ER-β) (Vandenberg et al., 2009). Meanwhile, both ER subtypes, the classical ER-α and newly identified ER-β, are expressed in cardiac fibroblasts (Grohe et al., 1997).

On the basis of above findings, we hypothesized that BPA, an estrogenic endocrine disruptor, could induce cardiac fibroblast proliferation and collagen production by activating estrogen receptor and/or the MAPK pathways. Thus, the present study was performed to verify the hypothesis and to explore its potential mechanisms with both in vivo and in vitro experimental approaches.

2. Materials and methods

2.1. Animal care

Healthy male Sprague-Dawley rats (150–200 g) were provided by the Experimental Animal Center of Harbin Medical University. Animals used in the current study were kept under standard animal room conditions (temperature 23 ± 1 °C, humidity 55 ± 5%) with a 12-h light and dark cycle. All rats were acclimated to the laboratory environment for 7 days before operation. The rats were randomly divided into four groups. The control group was gavaged with corn oil. BPA (5), BPA (20), and BPA (100) were gavaged with BPA (Sigma, St. Louis, MO) dissolved in corn oil respectively at dosages of 5, 20, 100 mg/kg/day for 30 consecutive days. Our study was approved by the ethic committees of Harbin Medical University. The protocols for animal handling were carried out in accordance with the Declaration of Helsinki and Institutional Animal Care and Use Committee of Harbin Medical University.

2.2. Echocardiography

A noninvasive transthoracic echocardiography was performed to evaluate the function of left ventricle as described previously (Xu et al., 2014). Briefly, after exposure to BPA for 30 consecutive days, rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.), and fixed on their backs with their fur shaved and skin cleaned. The parasternal long axis view was selected by using a high-frequency linear-array transducer. M-mode recordings were performed at the level of the papillary muscles to check the parameters of heart structure and function. The following parameters were obtained: interventricular septum (IVS), left ventricular internal dimension (LVID), left ventricular posterior wall (LVPW), ejection fraction (EF), and fractional shortening (FS).

2.3. Histochemistry and transmission electron microscopy

After transthoracic echocardiography had been obtained, rats were euthanized, and their hearts were removed rapidly and washed in ice-cold 0.9% saline. Parts of the heart were fixed in 4% paraformaldehyde, embedded in paraffin, and cross-sectionally cut