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BPA-induced DNA hypermethylation of the master mitochondrial gene PGC-1α contributes to cardiomyopathy in male rats

Ying Jiang [1], Wei Xia [1], Jie Yang, Yingshuang Zhu, Huailong Chang, Juan Liu, Wenqian Huo, Bing Xu, Xi Chen, Yuanyuan Li [*], Shunqing Xu [*]

Key Laboratory of Environment and Health, Ministry of Education & Ministry of Environmental Protection, and State Key Laboratory of Environmental Health (Incubating), School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

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ABSTRACT

Implication of environmental endocrine disruptors, such as bisphenol A (BPA), on the development of cardiopathy has been poorly investigated. The aim of the study was to investigate the effects of long-term exposure to BPA at the reference dose on the myocardium of rats, and the underlying mechanisms.

Male rats received corn oil or 50 μg/kg/day of BPA since delactation. At 24 and 48 weeks (wk), cardiac function and mitochondrial function were examined. The mRNA expression and the methylation status of PCG-1α, a major regulator of mitochondrial biogenesis in cardiac muscle, were also tested.

At 48 wk, BPA-exposed rats displayed cardiomyopathy, characterized by myocardium hypertrophy, cardiomyocyte enlargement, and impairment of cardiac function. At 24 wk, significantly reduced ATP production, dissipated mitochondrial membrane potential (Ψm) and declined mitochondrial respiratory complex (MRC) activity in cardiomyocytes were observed in BPA-exposed rats compared with the control rats, indicating a decrease in mitochondrial function occurs before the development of cardiomyopathy. Additionally, BPA exposure decreased the expression of PGC-1α and induced hypermethylation of PGC-1 α in heart tissue in 24- and 48-week-old rats. The change in methylation of PGC-1α was observed more pronounced in BPA-exposed rats at 48 wk.

Overall, long-term BPA exposure induces cardiomyopathy in male rats, and the underling mechanism may involve the impairment of cardiac mitochondrial function and the disturbance of methylation of PGC-1α.

1. Introduction

Bisphenol A (BPA) is a synthetic compound that is widely used in the manufacture of plastics and epoxy resins (Birnbaum et al., 2012). Owing to its widespread use in consumer products, such as canned foods, plastic products pressure-printed receipts and dental sealants, humans are exposed to BPA on a daily basis (Vandenberg et al., 2010). Some previous studies have indicated potential links between BPA exposure and chronic diseases, including obesity, cancer, diabetes and reproductive disorders (Diamanti-Kandarakis et al., 2009; Zoeller et al., 2012). Multiple longitudinal and cross-sectional and epidemiological studies have showed that in adults, BPA exposure levels are associated with heart diseases (Bae et al., 2012; Lang et al., 2008; Melzer et al., 2012; Shankar et al., 2012). These studies include several independent analyses of NHANES indicating that the participants'

Abbreviations: ATP5E, ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit; ATP5O, ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit; BPA, bisphenol A; CpG, cytosine phosphate guanine; DNMT3A, DNA methyltransferase 3A; DNMT3B, DNA methyltransferase 3B; DNMT1, DNA (cytosine-5-)-methyltransferase; LVID, d, end-diastolic left ventricular internal dimension; LVID, s, end-systolic left ventricular internal dimension; LVPW,s, left ventricle posterior wall thickness at systole; LVPW,d, left ventricle posterior wall thickness at diastole; MMP, mitochondrial membrane potential; MRC, mitochondrial respiratory complexes; NRF-1, nuclear respiratory factor 1; NRF-2, nuclear respiratory factor 2; PGC-1α, peroxisome proliferative-activated receptor coactivator 1 alpha; PPARα, peroxisome proliferator-activated receptor alpha; PPARγ, peroxisome proliferator-activated receptor gamma; TFAM, transcription factor A, mitochondrial; UQCRC2, cytochrome b-c1 complex subunit 2, mitochondrial; UQCRF1, ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1.

[*] Corresponding authors. Tel.: +86 27 83657705; fax: +86 27 83657781.
E-mail addresses: liyuanyuan@hust.edu.cn (Y. Li), xust@hust.edu.cn (S. Xu).

[1] Both authors contributed equally to this work.

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reported cardiac diagnoses were related with higher urinary BPA levels. No other types of diseases except diabetes were associated with BPA urine concentrations in these studies, which highlights that BPA exposure may be a potential risk factor for heart disease. In addition, animal studies have been carried on to explore the effects of long-term BPA exposure on cardiac system. Patel et al. (2013) demonstrated that lifelong BPA exposure altered cardiac function and structure in C57BL/6n mice in a sex-specific manner. A recent work by Chapalamadugu et al. provided evidences that BPA exposure in early gestational or late gestational period exposure have adverse impact on the offspring cardiac system. They found that maternal BPA exposure significantly altered fetal transcript expression profile in the fetal heart of primates, which were shown to be related to cardiac pathology such as cardiac hypertrophy (Chapalamadugu et al., 2014).

However, a recent study reported no association between higher urinary BPA levels and risk of heart attack, which questioned the suitability of causal associations (Lakind et al., 2014; LaKind et al., 2012). On the other hand, many studies have pointed out the limitations of the cross-sectional design (Adami et al., 2011; LaKind et al., 2012). The studies on BPA and cardiac outcome based on surveys such as NHANES, have nevertheless been regarded to support or contradict a link between exposure and risk of developing heart disease, and the role of BPA in heart disease remains unclear. In addition, the animal studies also showed inconsistent results about the effects of BPA exposure on cardiac impairment (Aboul Ezz et al., 2013; Delclos et al., 2014; Patel et al., 2013). Most of the experimental results were based on short-term exposure to BPA, but the investigation on the effects of long-term exposure to BPA on cardiovascular disease in vivo model and the underlying molecular mechanisms are limited.

Mitochondrial dysfunction plays a central role in the pathogenesis of cardiac diseases (Bayeva et al., 2013). BPA has been reported to induce the mitochondrial dysfunction in liver at the dose below the no observed adverse effect level (Moon et al., 2012), implying mitochondria is one of the target organelle of BPA. It is possible that BPA may dysregulate mitochondrial metabolism through specific regulatory pathway in the heart, and then ultimately lead to adverse cardiac effect.

Increasing studies suggest that epigenetic modifications provide a potential molecular basis for the interaction between environmental and genetic factors on energy metabolism, and may contribute to the manifestation of cardiac disease (Ozanne and Constancia, 2007). Epigenetic changes are not only important for understanding the molecular underpinning in environmental-related diseases, but also are regarded as biomarkers for toxicity assessment (Maranghi et al., 2010). Peroxisome proliferative-activated receptor coactivator 1 alpha (PGC-1α) is a major regulator of mitochondrial biogenesis in cardiac muscle (Arany et al., 2005), and plays a central role in the patho-mechanisms in myocardium hypertrophy and heart failure (Madrazo and Kelly, 2008; Ventura-Clapier et al., 2008). As a critical regulator of cardiac ATP generation and cardiac work (Handschin and Spiegelman, 2006; Sano and Schneider, 2005), PGC-1α is sensitive to environmental factors, such as environmental chemicals (Ling et al., 2004).

Therefore, in the present study, we determined the effects of long-term exposure to BPA at reference dose on the development of cardiopathy and cardiac mitochondrial function in male rats. The status of DNA methylation and expression of PGC-1α in the heart tissue were investigated. Furthermore, we also determined the expression of PGC-1 coactivated transcriptional factors and downstream genes involved in mitochondrial function to assess whether the PGC-1α transcriptional activity is compromised during the BPA exposure, which may help to illustrate the mechanism.

2. Material and methods

2.1. Maintenance and treatment of animals

All animal experiments in this study were conducted in accordance with experimental protocols that were approved by the Ethics Committee and followed the guidelines for the care and use of animals established by Tongji Medical College, Huazhong University of Science and Technology. The sample size was determined based on the data from preliminary experiment by power analysis described previously (Festing and Altman, 2002). In brief, we did a preliminary experiment about the effects of BPA on heart/body weight, which is a key point in the study, and then got the estimates for the means and standard deviations of heart/body weight for calculating the sample size for the present study. The power of the experiment was set to be 0.8 (we seek an 80% chance to find statistical significance if the specified effect exists), and the significance level was set to be 0.05. After the calculation, 8 rats in each group were required to provide statistical power (1-β) of 0.8 and a significant level of 0.05. Generally in the present study, the weaning (21 days after birth) male (60–80 g) Wistar rats (Hubei Research Center of Laboratory Animal, China) were housed in the special pathogen-free condition, with ad libitum access to water and food in an environmentally controlled room maintained on a 12-h light/dark cycle. Polypropylene cages and glass water bottles were used in this study. We have a total of 48 rats at the beginning of the experiment, the rats were numbered in random order and then allocated into two group (24 rats in the control group and 24 rats in BPA-exposed group) using a computer program. To be specific, we selected 8 rats in control and 8 rats in BPA-exposed group and another 8 rats as the standby at each time point (24 weeks and 48 weeks). Then, the two groups (control and BPA-exposed) were treated as follows: (i) the control group was fed with standard chow diet (STD); (ii) the BPA-group was fed with STD and treated with 50 μg/kg/day of BPA (Sigma, USA, purity ≥99%, CAS # 80-05-7). Dose selection in this experiment was based on the current USEPA's reference for safe daily limit (50 μg/kg bw/day, USEPA, 1993, http://www.epa.gov/iris/subst/0356.htm). The method of BPA treatment have been described previously (Fan et al., 2013): BPA was dissolved in corn oil (Sigma, USA, CAS # 8001-30-7) and diluted with three stock solutions (20, 40, and 60 mg/ml). The volumes of BPA stock solution were calculated based on the daily body weight (the volume limit <0.5 ml). Corn oil was added to the BPA stock solution to make a total volume of 0.5 ml, then directly mixed it with standard rodent chow (5 g/rat) as the BPA treatment diet. In the morning, the two group rats were provided with the BPA treatment diet or rodent chow diet containing the same volume of corn oil (0.5 ml), respectively until the age of 48 weeks (wk). In addition, sufficient chow diet was supplied if the treatment diet was completely consumed. The consumption of diets was calculated daily and the body weight was monitored weekly.

At the age of 24 and 48 weeks (wk), the rats were sacrificed, and blood was collected for BPA quantification. Before sacrifice of animals, the blood pressure was measured (8 rats) and echocardiographic assay (8 rats) was performed. After sacrifice of animals, the hearts were removed for the rest of analysis.

A summary of the experimental design is presented in Supplemental Fig. 1. All the rats were maintained in the same environment. In order to avoid the bias, all the experimental procedures were performed in a blind fashion that the samples and treatments were coded until the data were analyzed.

2.2. Blood pressure

Blood pressure was measured in conscious animals using a noninvasive system (Kent Scientific, Torrington, CT, USA) by the tail