Environmental Research 147 (2016) 324–330

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Modification of the association of bisphenol A with abnormal liver function by polymorphisms of oxidative stress-related genes

Jin Hee Kim [a,b], Mee-Ri Lee [c], Yun-Chul Hong [c,d,*]

[a] Department of Environmental Health, Graduate School of Public Health, Seoul National University, Seoul 151-742, Republic of Korea
[b] Department of Bioscience and Bioengineering, Sejong University, Seoul, 05006, Republic of Korea
[c] Department of Preventive Medicine, Seoul National University College of Medicine, Seoul 110-799, Republic of Korea
[d] Institute of Environmental Medicine, Seoul National University Medical Research Center, Seoul 110-799, Republic of Korea

ARTICLE INFO

Article history:
Received 28 October 2015
Received in revised form
26 January 2016
Accepted 18 February 2016

Keywords:
Bisphenol A
Liver function
Oxidative stress
Genetic polymorphisms

ABSTRACT

Some studies suggested oxidative stress as a possible mechanism for the relation between exposure to bisphenol A (BPA) and liver damage. Therefore, we evaluated modification of genetic polymorphisms of cyclooxygenase 2 (*COX2* or *PTGS2*), epoxide hydrolase 1 (*EPHX1*), catalase (*CAT*), and superoxide dismutase 2 (*SOD2* or *MnSOD*), which are oxidative stress-related genes, on the relation between exposure to BPA and liver function in the elderly. We assessed the association of visit-to-visit variations in BPA exposure with abnormal liver function by each genotype or haplotype after controlling for age, sex, BMI, alcohol consumption, exercise, urinary cotinine levels, and low density lipoprotein cholesterol using a GLIMMIX model. A significant association of BPA with abnormal liver function was observed only in participants with *COX2* GG genotype at rs5277 (odds ratio (OR)=3.04 and p=0.0231), *CAT* genotype at rs769218 (OR=4.16 and p=0.0356), *CAT* CT genotype at rs769217 (OR=4.19 and p=0.0348), *SOD2* TT genotype at rs4880 (OR=2.59 and p=0.0438), or *SOD2* GG genotype at rs2758331 (OR=2.57 and p=0.0457). Moreover, we also found higher OR values in participants with a pair of G-G haplotypes for *COX2* (OR=2.81 and p=0.0384), G-C-A haplotype for *EPHX1* (OR=4.63 and p=0.0654), A-T haplotype for *CAT* (OR=4.48 and p=0.0245), or T-G-A haplotype for *SOD2* (OR=2.91 and p=0.0491) compared with those with the other pair of haplotypes for each gene. Furthermore, the risk score composed of 4 risky pair of haplotypes showed interactive effect with BPA on abnormal liver function (p=0.0057). Our study results suggest that genetic polymorphisms of *COX2*, *EPHX1*, *CAT*, and *SOD2* modify the association of BPA with liver function.

1. Introduction

Bisphenol A (BPA) is one of the highest volume chemicals produced worldwide, which increases by 6–10% annually (Vandenberg et al., 2007). Because BPA is employed to make polycarbonate plastics and epoxy resins used in a variety of common consumer products including water pipes and beverage cans (Bushnik et al., 2010; Hanioka et al., 2008; vom Saal et al., 2005), human are ubiquitously exposed to it. BPA acts like hormone to interrupt balance of endocrine system in the body, leading to early sexual maturation (Howdeshell et al., 1999), elevated incidence of reproductive organ lesions (Newbold et al., 2007), prostate developmental abnormalities (Timms et al., 2005), and decreased sperm production (vom Saal et al., 2005). The ubiquitous exposure of BPA and its toxic potential raise concerns about its effects on non-sexual organs as well (Korkmaz et al., 2010; Lang et al., 2008; Lee et al., 2014). Recently, several studies suggested adverse health effects on liver function by BPA exposure. A study on BPA-treated rats showed elevated aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) levels as well as marked defects in liver morphology (Korkmaz et al., 2010). Moreover, a cross-sectional study of 1455 Americans demonstrated a significant association between higher urinary BPA and abnormal concentrations of three liver enzymes: γ-glutamyl transferase (γ-GTP), alkaline phosphatase (ALP), and LDH

Abbreviations: BPA, bisphenol A; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; γ-GTP, γ-glutamyl transferase; ALP, alkaline phosphatase; COX2, cyclooxygenase 2; EPHX1, epoxide hydrolase 1; CAT, catalase; SOD2, superoxide dismutase 2; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; MAF, minor allele frequency; LOD, limit of detection; LD, linkage disequilibrium; D′, relative disequilibrium; BMI, body mass index; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; CI, confidence interval; ICC, intraclass correlation coefficient

* Corresponding author at: Department of Preventive Medicine, Seoul National University College of Medicine, 103 Daehakro, Jongro-gu, Seoul 110-799, Republic of Korea.
E-mail address: ychong1@snu.ac.kr (Y.-C. Hong).

http://dx.doi.org/10.1016/j.envres.2016.02.026
0013-9351/

(Lang et al., 2008). Furthermore, the adverse effect of BPA exposure on liver enzymes, AST, ALT, and γ-GTP was also found in our previous longitudinal elderly panel study (Lee et al., 2014).

Some studies suggested oxidative stress as a possible mechanism for the relation between exposure to BPA and liver damage (Hassan et al., 2012; Korkmaz et al., 2010; Moon et al., 2012). If oxidative stress is a major biologic pathophysiological mechanism underlying the effect of BPA in regard of liver function, variations of oxidative stress-related genes may affect the relation between BPA exposure and liver function. Cyclooxygenase 2 (*COX2* or *PTGS2*), epoxide hydrolase 1 (*EPHX1*), catalase (*CAT*), and superoxide dismutase 2 (*SOD2*, *MnSOD*) are oxidative stress-related genes. COX2 is the key enzyme in prostaglandin biosynthesis acting both as a dioxygenase and as a peroxidase (O'Banion, 1999). EPHX1 is a critical biotransformation enzyme that converts epoxides from the degradation of aromatic compounds to trans-dihydrodiols which can be conjugated and excreted from the body (Decker et al., 2009). CAT is a key antioxidant enzyme converting hydrogen peroxide to water and oxygen, and thereby mitigating the toxic effects (Liu et al., 2015; Rupérez et al., 2013). SOD2 catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide (Fukai et al., 2011). In fact, several reports showed relations of their polymorphisms with oxidative stress or liver diseases such as hepatocellular carcinoma and hepatic inflammation and fibrosis (Al-Serri et al., 2012; Bošković et al., 2013; Bresciani et al., 2013; Bu et al., 2013; Gharib et al., 2014; Komina et al., 2012; Lakhdar et al., 2011; Miyashita et al., 2012; Nahon et al., 2009; Vibhuti et al., 2007; Zhong et al., 2013).

Therefore, in this study, we evaluated the effect of *COX2*, *EPHX1*, *CAT*, and *SOD2* genetic polymorphisms on the relation between exposure to BPA and liver function in the elderly. If these oxidative stress-related genes are involved in the toxicological pathway of liver dysfunction by BPA, it may support the pathogenic role of oxidative stress on liver by BPA.

2. Materials and methods

2.1. Study population and sampling

Lee et al. (2014) evaluated the association between BPA exposure and abnormal liver function in the elderly aged 60 or over recruited from the Korean Elderly Environmental Panel (KEEP) study. This study was conducted as a subsequent action of the previous study to estimate the effect of oxidative stress-related gene polymorphisms on the association. Briefly, a total of 560 elderly people visited a community elderly welfare center as many as five times for medical examination and urine collection (twice in 2008, once in 2009 and twice in 2010), but fasting blood samples were collected 3 times (once every year) (Lee et al., 2014). Among 560 elderly subjects, 25 whose blood and urine samples were unavailable, 52 who visited the center only once, 6 who currently had any type of viral hepatitis, fatty liver disease, liver cancer, any other liver disease, or high level of triglyceride (>800 mg/dL), and 6 who had repeated high levels of BUN-creatinine ratio (>30) as a renal failure criteria were excluded, and finally 471 subjects and 1000 sample pairs with both urine and blood were included in the analysis.

2.2. BPA measurement

We measured urinary levels of total BPA, including free and conjugated BPA, using HPLC tandem mass spectrometry (HPLC: Agilent 1200, USA; MS/MS: Agilent 6410 Triple Quad LCMS, Agilent, USA) according to the previously reported procedures (Lee et al., 2014). For BPA measurement, five-hundred microliters of urine were buffered with 30 μL of 2.0 m sodium acetate (pH 5.0), and then spiked with 25 μL internal standard BPA (RING-13C12, 99%; Cambridge Isotope Lab, Inc., Andover, MA, USA) and 10 μL of glucuronidase/sulfatase (Sigma–Aldrich, St. Louis, MO, USA). Accuracy, coefficient of precision variation, and coefficient of reproducibility variation were 99.7%, 1.0–4.7, and 0.5–5.3, respectively, based on the quality control method adopted from the Clinical and Laboratory Standards Institute (CLSI) guideline.

2.3. Liver enzymes measurement

Blood samples (up to 3 mL) were collected from each participant and serum levels of AST, ALT, and γ-GTP, hepatocellular injury markers, for a criteria of abnormal liver function were measured according to the previously reported procedures (Lee et al., 2014).

2.4. Total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C)

Serum levels of TC, TG, and high-density lipoprotein cholesterol (HDL-C) were measured by an autobiochemical analyser (Hitachi 7600-II, Hitachi High-Technologies, Japan) with reagents Pureauto S CHO-N (Daiichi Pure Chemicals, Tokyo, Japan), Pureauto S TG-N (Daiichi Pure Chemicals, Tokyo, Japan), and CHOLESTEST N HDL (Daiichi Pure Chemicals, Tokyo, Japan), and then we calculated LDL-C levels according to the following equation: TC (mg/dL) – HDL-C (mg/dL) – [TG (mg/dL)/5.0] (Friedewald et al., 1972). Calculated LDL-C levels with minus value were assigned as zero.

2.5. Creatinine measurement

For dilution correction in the analyses, creatinine concentration was measured according to the previously reported procedure (Lee et al., 2014).

2.6. Cotinine measurement

Urinary cotinine levels were measured for monitoring tobacco exposure. Cotinine level was analyzed by an enzyme-linked immunosorbent assay method (Nahon et al., 2009).

2.7. Genotyping of COX2, EPHX1, CAT, and SOD2

To estimate the effect of genetic polymorphisms of *COX2*, *EPHX1*, *CAT*, and *SOD2* on the relation between BPA exposure and abnormal liver function, firstly we selected twelve target SNPs (rs20417, rs5277, and rs3218625 for *COX2*, rs3766934, rs1051740, and rs2234922 for *EPHX1*, rs769218 and rs769217 for *CAT*, and rs4880, rs2842957, rs2758331, and rs5746136 for *SOD2*) based on more than 0.1 minor allele frequency (MAF) for Han Chinese in Beijing and Japanese in Tokyo obtained from HapMap or functional association with diseases published in PubMed. However, in our pilot genotyping with our samples, rs20417 was found to have 0% MAF (all GG genotype) and rs2842957 was not genotyped due to nearby similar sequence. Therefore finally we selected a total of ten SNPs including 2 SNPs for *COX2* (rs5277 and rs3218625), 3 SNPs for *EPHX1* (rs3766934, rs1051740, and rs2234922), 2 SNPs for *CAT* (rs769218 and rs769217), and 3 SNPs for *SOD2* (rs4880, rs2758331, and rs5746136) for genotyping.

Genomic DNA was extracted from peripheral blood lymphocytes using a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) and ten polymorphisms of *COX2*, *EPHX1*, *CAT*, and *SOD2* genes were determined using the TaqMan fluorogenic 5′ nuclease assay (rs5277 for *COX2*, rs1051740, rs2234922, and rs3766934 for *EPHX1*, rs769218 and rs769217 for *CAT*, and rs4880, rs2758331, and rs5746136 for *SOD2*) and single base primer extension assay