Environmental Research 150 (2016) 573–581

Contents lists available at ScienceDirect

Environmental Research

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

Urinary biomarkers of exposure to 57 xenobiotics and its association with oxidative stress in a population in Jeddah, Saudi Arabia

Alexandros G. Asimakopoulos [a,1], Jingchuan Xue [a,f,1], Bruno Pereira De Carvalho [a], Archana Iyer [b], Khalid Omer Abualnaja [c], Soonham Sami Yaghmoor [d], Taha Abdullah Kumosani [e], Kurunthachalam Kannan [a,e,f,*]

[a] Wadsworth Center, New York State Department of Health, Empire State Plaza, P.O. Box 509, Albany, New York 12201-0509, United States
[b] Biochemistry Department, Faculty of Science, Production of Bioproducts for Industrial Applications Research Group, Vitamin D Pharmacogenomics Research Group and Experimental Biochemistry Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia
[c] Biochemistry Department, Faculty of Science and Bioactive Natural Products Research Group, King Abdulaziz University, Jeddah, Saudi Arabia
[d] Experimental Biochemistry Unit, King Fahd Medical Research Center and Production of Bioproducts for Industrial Applications Research Group, King Abdulaziz University, Jeddah, Saudi Arabia
[e] Biochemistry Department, Faculty of Science, Production of Bioproducts for Industrial Applications Research Group and Experimental Biochemistry Unit, King Fahd Medical Research, Center King Abdulaziz University, Jeddah, Saudi Arabia
[f] Department of Environmental Health Sciences, School of Public Health, State University of New York at Albany, Albany, New York, United States

ARTICLE INFO

Article history:
Received 4 August 2015
Received in revised form
12 November 2015
Accepted 23 November 2015
Available online 3 December 2015

Keywords:
Phthalates
Phenolics
Parabens
Oxidative stress
Biomonitoring

ABSTRACT

Oxidative stress arises from excessive free radicals in the body and is a trigger for numerous diseases, such as cancer and atherosclerosis. Elevated exposure to environmental chemicals can contribute to oxidative stress. The association between exposure to xenobiotics and oxidative stress, however, has rarely been studied. In this study, urinary concentrations of 57 xenobiotics (antimicrobials, parabens, bisphenols, benzophenones, and phthalates metabolites) were determined in a population from Jeddah, Saudi Arabia, to delineate association with the oxidative stress biomarker, 8-hydroxy-2′-deoxyguanosine (8OHDG). We collected 130 urine samples and analyzed for 57 xenobiotics using liquid chromatography-tandem mass spectrometry (LC/MS/MS) methods. The association between unadjusted and creatinine- or specific gravity-adjusted concentrations of xenobiotics and 8OHDG was examined by Pearson correlations and multiple regression analysis. High concentrations of mCPP (a metabolite of di-n-octyl phthalate; DnOP) and mCMHP (a metabolite of diethylhexyl phthalate; DEHP) were found in urine. In addition, the concentrations of bisphenol S (BPS) were higher than those of bisphenol A (BPA). The concentrations of metabolites of DEHP, phthalic acid, BPA, BPS, and methyl-protocatechuic acid were significantly associated with 8OHDG. This is the first biomonitoring study to report exposure of the Saudi population to a wide range of environmental chemicals and provides evidence that environmental chemical exposures contribute to oxidative stress.

1. Introduction

Oxidative stress is a condition that arises from an imbalance in the redox state and an overload of reactive oxygen species in cells and tissues. Oxidative stress can disrupt normal cellular signaling and can act as a trigger for numerous diseases, such as cancer, infertility, and Alzheimer's. Urinary concentrations of 8-hydroxy-2′-deoxyguanosine (8OHDG) have been reported as a biomarker of oxidative stress (Ravanat et al., 1998). Oxidation of DNA occurs normally but that increases with elevated exposure to oxidizing agents (Guo et al., 2014). Exposure to a range of synthetic environmental chemicals can augment oxidative damage to DNA. Exposure of humans to environmental chemicals, such as antimicrobials, *p*-hydroxybenzoic acid esters (parabens; preservatives), bisphenols (BPs; intermediates in the production of epoxy resins and polycarbonate plastics), benzophenone-type UV filters (BzPs; sunscreen agents); bisphenol A diglycidyl ethers (BADGEs; industrial ethers), bisphenol F diglycidyl ethers (BFDGEs; industrial ethers), novolac glycidyl ethers (NOGEs; industrial ethers), phthalates (plasticizers), and benzothiazoles/benzotriazoles (BTHs/BTRs; anti-corrosive agents), has been reported in populations from around the world (Asimakopoulos et al., 2012, 2013a, b, 2014a,b; Asimakopoulos and Thomaidis,

* Corresponding author at: Wadsworth Center, Empire State Plaza, P.O. Box 509, Albany, NY 12201-0509. Fax: +1 518 473 2895.
E-mail address: kurunthachalam.kannan@health.ny.gov (K. Kannan).
[1] Co-first author contributed to this study equally.

http://dx.doi.org/10.1016/j.envres.2015.11.029
0013-9351/

2015; Callan et al., 2012; Casas et al., 2011; Colacino et al., 2010; Fisher et al., 2015; Frederiksen et al., 2013, 2014; Goldstone et al., 2015; Geens et al., 2014; Guo et al., 2014; Högberg et al., 2008; Itoh et al., 2007, 2009; Koch et al., 2003; López-Carrillo et al., 2010, Liao and Kannan, 2012; Liao et al., 2012; Philippat et al., 2012; Quirós-Alcalá et al., 2013; Wang et al., 2013; Xue et al., 2015; Ye et al., 2008; Zhang et al., 2011; Zhou et al., 2014; Ji et al., 2010). However, the relationship between exposure to environmental xenobiotics and oxidative stress is not well established.

Analysis of trace levels of environmental chemicals in human specimens for the establishment of association between exposure and effect biomarkers can be challenging. For population-based biomonitoring studies, a time- and cost-effective method for the analysis of human specimens, without compromising the data quality, is required. Further, the data analysis should take into consideration the methods to analyze censored data (i.e., non-detects; NDs). The use of appropriate statistical models is required to establish the relationship between chemical exposures in populations and biomarkers of adverse health effects. In particular, appropriate methods to analyze censored data continue to be a challenging issue. With this background, the present study aimed to establish urinary levels (total concentrations) of 57 xenobiotics in a general population from Jeddah, Saudi Arabia, to assess exposures and to delineate the association with oxidative stress in that population. We examined inter-correlations between xenobiotics and the association between xenobiotics and 8OHDG.

2. Materials and methods

Urine samples were analyzed for two antimicrobials (triclosan, TCS; and triclocarban, TCC), 10 parabens (methyl-, MeP; ethyl-, EtP; propyl-, PrP; butyl-, BuP; benzyl-, BzP; heptyl-paraben, HeP; 4-hydroxy benzoic acid, 4HB; 3,4-dihydroxy benzoic acid, 3,4DHB; methyl-protocatechuic acid, OHMeP; and ethyl-protocatechuic acid, OHEtP), eight BPs (2,2-bis(4-hydroxyphenyl)propane, BPA; 4,4′-(hexafluoroisopropylidene)-diphenol, BPAF; 4,4′-(1-phenylethylidene)bisphenol, BPAP; 4,4′-sulfonyldiphenol, BPS; 4,4′-dihydroxydiphenylmethane, BPF; 4,4′-(1,4-phenylenediisopropylidene)bisphenol, BPP; 4,4′-cyclo-hexylidenebisphenol, BPZ; and 2,2-bis(4-hydroxyphenyl)butane, BPB), five BzPs (2-hydroxy-4-methoxybenzophenone, BP3; 2,4-dihydroxybenzophenone, BP1; 2,2′-dihydroxy-4-methoxybenzophenone, BP8; 2,2′,4,4′-tetrahydroxybenzophenone, BP2; and 4-hydroxybenzophenone, 4OHBP), six BADGEs (bisphenol A diglycidyl ether, BADGE; bisphenol A (2,3-dihydroxypropyl) glycidyl ether, BADGE · H_2O; bisphenol A (3-chloro-2-hydroxypropyl) glycidyl ether, BADGE · HCl; bisphenol A bis(2,3-dihydroxypropyl) glycidyl ether, BADGE · $2H_2O$; bisphenol A bis(3-chloro-2-hydroxypropyl) glycidyl ether, BADGE · 2HCl; and bisphenol A (3-chloro-2-hydroxypropyl) (2,3-dihydroxypropyl) glycidyl ether, BADGE · H_2O · HCl), three BFDGEs (bisphenol F diglycidyl-ether, BFDGE; bisphenol F bis(3-chloro-2-hydroxypropyl)glycidylether, BFDGE · 2HCl; and bisphenol F bis(2,3-dihydroxypropyl)glycidylether, BFDGE · $2H_2O$), 2 NOGEs (3-ring novolac glycidyl ether, 3RNOGE; and 4-ring novolac glycidyl ether, 4RNOGE), 18 phthalates metabolites (mono-(2-ethyl-5-carboxypentyl) phthalate, mECPP; mono-[(2-carboxymethyl) hexyl] phthalate, mCMHP; mono-(2-ethyl-5-oxohexyl) phthalate, mEOHP; mono-(2-ethyl-5-hydroxyhexyl) phthalate, mEHHP; mono-(3-carboxypropyl) phthalate, mCPP; mono-2-isobutyl phthalate, mIBP; mono-cyclohexyl phthalate, mCHP; mono-isononyl phthalate, mINP; phthalic acid, PA; mono-(8-methyl-1-nonyl) phthalate, mIDP; mono-octyl phthalate, mOP; mono-n-butyl phthalate, mBP; mono-hexyl phthalate, mHxP; mono-2-heptyl phthalate, mHpP; mono-methyl phthalate, mMP; mono-ethyl phthalate, mEP; mono-benzyl phthalate, mBzBP; and mono-(2-ethylhexyl) phthalate, mEHP), two BTHs (benzothiazole, BTH; and 2-hydroxy-benzothiazole, 2OHBTH), one BTR (xylyltriazole, XTR), 8OHDG, specific gravity (SG), and creatinine (CR).

The urine samples were collected from 130 individuals from the general population in Jeddah, Saudi Arabia, in May and June 2014. The samples were collected from healthy individuals who visited King Abdulaziz University Hospital for routine examination. Of the 130 samples collected, age and gender information were available for 67 individuals (31 males and 36 females; 63 unknown). The ages of donors ranged from 1 to 87 years with a median value of 35 years (mean: 37 years, standard deviation: 25). All samples were stored at −20 °C until analysis. The study was approved by the Institutional Review Boards of Wadsworth Center, New York State Department of Health, and King Abdulaziz University, Jeddah, Saudi Arabia. For the preparation of matrix-matched calibration curve, pooled urine samples were obtained by mixing equal volumes of urine from 6 individuals (3 male and 3 female donors).

Urine samples were extracted using liquid-liquid extraction (LLE) (Asimakopoulos et al., 2014a,b; Xue et al., 2015) for the analysis of all xenobiotics listed above, except for phthalates metabolites (Fig. S1). The chromatographic separation of parabens and antimicrobials was carried out using a Waters Acquity™ ultra performance liquid chromatography (UPLC) system (Waters, Milford, MA, U.S.), which consisted of a binary pump and an auto sampler. Identification and quantification of target analytes were accomplished with an Applied Biosystems API 5500™ electrospray triple quadrupole mass spectrometer (ESI–MS/MS; Applied Biosystems, Foster City, CA, U.S.) under the negative ionization mode. A Kinetex C18 column (2.1 mm × 50 mm, 1.3 μm; Phenomenex Inc., Torrance, CA, U.S.) serially connected to a SecurityGuard ULTRA C18 guard column (2.1 mm, sub-2 μm core-shell column; Phenomenex Inc.) was used for separation of target chemicals. The mobile phase comprised methanol (A) and Milli-Q water that contained 1% (v/v) formic acid (B). Low limits of detection (LODs) on the order of sub-nanogram per liter (ppt) were obtained **(see** Table 1 **for details)**.

The chromatographic separation of BPs and BzPs was achieved using a Shimadzu Prominence™ Modular HPLC system (Shimadzu Corporation, Kyoto, Japan). Identification and quantification of BPs and BzPs were performed with an API 3200™ electrospray triple quadrupole mass spectrometer (ESI-MS/MS; Applied Biosystems) under the negative ionization mode. A Betasil C18 column (2.1 mm × 100 mm, 5 μm; Thermo Electron Corp., Waltham, MA, U.S.) serially connected to a Javelin guard column (Betasil C18, 2.1 mm × 20 mm, 5 μm; Thermo Electron Corp.) was used for separation. The mobile phase comprised methanol (A) and Milli-Q water that contained 0.1% (v/v) ammonium hydroxide (B). The use of ammonium hydroxide in the mobile phase and periodic injection of acetone (as a blank solvent) in the LC-MS/MS system were critical to enhancing instrumental sensitivity and signal stability. The use of ammonium hydroxide in the mobile phase enabled BPA analysis possible with a simple LLE sample preparation. The LODs for BPs and BzPs were in the ranges of 0.035 (BPB)–0.57 (BPF) ng/mL and 0.004 (BP1) –0.28 (BP3) ng/mL, respectively (Table 1).

The chromatographic separation of BTHs and XTR was performed using the instrument as described above for BPs and BzPs with methanol (A) and Milli-Q water (B) as mobile phases. The chromatographic separation of BADGEs, BFDGEs, and NOGEs was carried out using an Agilent 1100 Series HPLC system (Agilent Technologies Inc., Santa Clara, CA, U.S.). Identification and quantification of BADGEs, BFDGEs, and NOGEs were performed with an Applied Biosystems API 2000™ ESI-MS/MS under the positive ionization mode, and the chromatographic column used was similar to that described for BPs and BzPs. The mobile phase comprised methanol (A) and Milli-Q water/Methanol (90:10, % v/v) that