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A study on phthalate metabolites, bisphenol A and nonylphenol in the urine of Chinese women with unexplained recurrent spontaneous abortion ☆

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ABSTRACT

Humans are widely exposed to phthalates, bisphenol A and nonylphenol owing to the ubiquitous use of these chemicals in consumer products. Increasing attention has been paid to exposure to phthalates, bisphenol A and nonylphenol because of their potential adverse effects on human fertility. A validated method was developed to investigate the three classes of environmental estrogen, mentioned above, in the urine of Chinese women of Nanjing area with unexplained recurrent spontaneous abortion. Solid-phase extraction coupled with ultra performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) was used. In this method, amounts of bisphenol A (BPA), nonylphenol (NP) and four phthalate metabolites, mono-n-butyl phthalate (MBP), mono-isobutyl phthalate (MiBP), mono-benzyl phthalate (MBzP) and mono-2-ethylhexyl phthalate (MEHP), along with their isotope labeled internal standards, were measured using UPLC-MS/MS operated in negative electrospray ionization multiple reaction monitoring mode. The limits of detection were 0.3 ng/mL for the four phthalate metabolites, and 0.5 ng/mL for bisphenol A and nonylphenol. For women with unexplained recurrent spontaneous abortion, the mean concentrations of MBP, MiBP, MBzP, MEHP, BPA and 4-n-NP were 6.52 ± 6.04, 5.51 ± 4.19, 0.53 ± 0.42, 10.12 ± 4.16, 7.13 ± 7.42, 0.41 ± 0.49 ng/mL (mean $\pm$ SD), respectively. For the control group, the mean concentrations of the corresponding analytes were 4.15 ± 3.57, 2.96 ± 3.30, 0.46 ± 0.49, 6.50 ± 2.81, 4.43 ± 2.23,0.48 ± 0.43 ng/mL (mean $\pm$ SD), respectively. Levels of MiBP and MEHP were significantly different between the two groups, using Wilcoxon rank sum tests. This method can be applied in epidemiological studies to explore the association between exposure to environmental estrogens and relevant adverse outcomes.

1. Introduction

Recurrent spontaneous abortion (RSA), defined as three or more pregnancy losses with the same partner before 20 weeks of gestation, affects 0.5–3% of couples worldwide (Li et al., 2002). Approximately 50% of RSA cases are of unknown cause, and these cases are referred to as unexplained RSA. More attention has been paid to the relationship between environmental factors and reproduction as the adverse effects of environmental contaminants on human health have been realized. Increasing numbers of studies suggest that environmental factors may be one of the causes of RSA, in addition to causes such as anatomical, thrombophilic and hormonal conditions, and gene mutations (Pandey et al., 2005; Zhang et al., 2010; Naderi-Mahabadi et al., 2015). Data from relevant studies are still quite limited, particularly for unexplained RSA. Hence, exploration of the relationship between exposure to environmental contaminants and unexplained RSA will be a vital research area in the future.

Environmental endocrine disruptors (EEDs) are environmental

Abbreviations: MPAEs, phthalate metabolites; MBP, mono-n-butyl phthalate; MiBP, mono-isobutyl phthalate; MBzP, mono-benzyl phthalate; MEHP, mono-2-ethylhexyl phthalate; BPA, bisphenol A; NP, nonylphenol; UPLC-MS/MS, ultra performance liquid chromatography–tandem mass spectrometry; SPE, solid-phase extraction; QC, quality control; INMA, Infancia y Medio Ambiente; MIREC, Maternal-Infant Research on Environmental Chemicals; CCCEH, Columbia Center for Children's Environmental Health; CEHS, Children's Environmental Health Study

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contaminants that disturb normal endocrine function and cause reproductive dysfunction in humans and other animals. Environmental estrogens are one of the key foci of studies on EEDs. The number of reports of adverse effects on human fertility of environmental estrogens increases each year.

Phthalates are common chemicals widely used in plastic containers, personal care products, food packages and so on (Arbuckle et al., 2014a). Bisphenol A (BPA) is a primary raw material in plastics and resins, and is commonly used in a variety of consumer products including paper products, water bottles and the internal lining of cans (Calafat et al., 2008; Newbold et al., 2007; Mei et al., 2012), whereas nonylphenols (NP) are commonly used in the production of elasticizers, technical grade abstergents and pesticide emulsifiers (Xiao et al., 2006). The exposure of humans to phthalates, BPA and NP has been considered to be ubiquitous, and may lead to severe damage to human health, especially to the reproductive system. For the general population, oral exposure has been considered the major route, and includes breathing contaminated air, ingestion of food, and incidental ingestion of soil or dust, among which food represents the most important source of exposure to phthalates, BPA and NP (Itoh et al., 2007; Franco et al., 2007; Herrero et al., 2015).

Phthalates, BPA and NP are well known as endocrine disruptors or environmental estrogens that are involved in widespread human exposure. Numerous studies have reported that these chemicals may cause reproductive effects, birth defects, endocrine disruption or even cancer (Mankidy et al., 2013; Ventrice et al., 2013; Asimakopoulos et al., 2012; Vandenberg et al., 2007; Dekant and Völkel, 2008; Lenie et al., 2008; Lyche et al., 2009). These chemicals have commonly been measured in urine in population surveys; however, such data are limited for women of childbearing age. Given the concern regarding phthalates, BPA and NP as substances produced and used in high amounts and in a wide range of consumer products, as well as their permanent existence in the environment and adverse effects on human fertility, determination of human exposures to these chemicals is of high importance, especially for women of childbearing age.

On the basis of the considerations above, solid-phase extraction (SPE) coupled with ultra performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) was established and assessed to detect four phthalate metabolites, mono-n-butyl phthalate (MBP), mono-isobutyl phthalate (MiBP), mono-benzyl phthalate (MBzP) and mono-2-ethylhexyl phthalate (MEHP), in addition to BPA and 4-n-NP, in human urine. The method was used to determinate the concentrations of the four phthalate monoesters, BPA and NP in the urine of women with unexplained RSA and a control group. This study aimed to explore the distribution of the target analytes among women of childbearing age and to evaluate a possible correlation between the levels of environmental estrogens in the human body and unexplained RSA.

2. Experimental methods

2.1. Reagents and materials

The MBP, MiBP, MBzP, MEHP, BPA and 4-n-NP were purchased from Accustandard (New Haven, CT, USA). The isotope labeled internal standards (IS) for D_4-mono-n-butyl phthalate (D_4-MBP), D_4-mono-benzyl phthalate (D_4-MBzP), D_4-mono-2-ethylhexyl phthalate (D_4-MEHP) and 4-n-NP-D_4 were purchased from C/D/N Isotopes (Quebec, Canada). The D_{16}-BPA was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). High-performance liquid chromatography (HPLC)-grade ammonium acetate, sodium dihydrogen phosphate dihydrate, phosphoric acid, ammonium hydroxide (> 25% in H_2O), formic acid, acetonitrile and methanol were purchased from TEDIA (Ohio, USA). Milli-Q water (Millipore, Bedford, MA, USA) was used throughout the study. β-Glucuronidase/sulfatase (β-glucuronidase from abalone > 10,0000 units/mL, arylsulfatase < 20,000 units/mL) was purchased from ANPEL Laboratory Technologies (Shanghai, China). The ABS ELUT-Nexus SPE cartridges (60 mg, 3 mL) were purchased from Agilent Technologies (Santa Clara, CA, USA). Frozen human urine samples (pooled from 60 individuals) were stored at −80 °C until use. All the glassware used in the experiments was methanol-rinsed and dried.

2.2. Instrumentation

The UPLC-MS/MS analysis was performed using a Thermo system, which consisted of a Thermo Accela LC system connected to a TSQ Quantum Access MAX triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) interface. Operation control and data processing were achieved by Thermo Xcalibur software.

2.3. UPLC conditions

The chromatographic separation was performed on a Waters Acquity UPLC BEH Phenyl column (1.7 μm, 2.1 mm × 100 mm, Waters, Milford, MA, USA) maintained at 30 °C with a Waters Vanguard precolumn. The sample temperature was set at 10 °C and the sample injection volume was 5 μL. Two analytical methods were used for phthalate metabolites, BPA and NP. The mobile phase for phthalate metabolites was 0.1% formic acid in water (A) and methanol (B) at a flow rate of 300 μL/min. The mobile phase gradient was as follows: 0–4 min, 30% B; 8 min, 40% B; 10 min, 50% B; 16–20 min, 70% B; 20.1–22 min, 30% B. The mobile phase for BPA and NP was 0.05% ammonia in water (A) and methanol (B), also at a flow rate of 300 μL/min. The mobile phase gradient was as follows: 0–2 min, 20% B; 6 min, 50% B; 8–12 min, 95% B; 12.1–14 min, 20% B. The retention time of MBP, MiBP, MBzP, MEHP, BPA and 4-n-NP was 13.0, 12.68, 13.99, 18.32, 8.73 and 9.96 min, respectively.

2.4. Mass spectrometer conditions

The electrospray probe was operated in the negative ion mode for both analytical methods. All analytes were quantified using multiple reaction monitoring (MRM) mode. The selected parameters were as follows: capillary voltage: 3.0 kV; spray voltage: 2.6 kV; vaporizer temperature: 380 °C; desolvation temperature: 350 °C. The MRM transitions monitored were as follows: MBP (precursor ion → product ion, m/z 221 → 77), MiBP (m/z 221 → 77), D_4-MBP (m/z 225 → 81), MBzP (m/z 255 → 183), D_4-MBzP (m/z 259 → 187), MEHP (m/z 277 → 134), D_4-MEHP (m/z 281 → 237), BPA (m/z 227 → 212), D_{16}-BPA (m/z 241 → 142), 4-n-NP (m/z 219 → 106), 4-n-NP-D_4 (m/z 223 → 110).

2.5. Blank controls

Given the ubiquitous presence of target analytes in the environment, all the glassware used in the experiments was rinsed consecutively with methanol and ultrapure water twice each before use. Blank samples were prepared with ultrapure water instead of human urine. The levels of all target analytes in the blank samples were measured and used as the baseline values for blank controls. In the validation study, “blank samples” were prepared by mixing urine samples from six individuals.

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