Associations of prenatal exposure to phenols with birth outcomes

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1. Introduction

Phenols are extensively used as a major component in a wide spectrum of products in industry, agriculture and daily life. Bisphenol A (BPA) has been used for over 50 years in the manufacture of polycarbonate plastics, epoxy resins and thermal paper (Centers for Disease Control and Prevention, 2009). The sunscreen benzophenone-3 (BP-3) is commonly used in lotions, conditioners, cosmetics and plastic surface coatings (Centers for Disease Control and Prevention, 2009). Alkylphenols (APs) [octylphenols (OPs) and nonylphenol (NPs)] are degradation products of the corresponding alkylphenol ethoxylates (APEs) which are surfactants used in detergents, industrial cleaners and emulsifiers (Bonefeld-Jorgensen et al., 2007; Centers for Disease Control and Prevention, 2009). The huge volume production and widespread application of the above compounds lead to the exposure to human. Recent studies in the fields of exposure science and analytical chemistry have documented that phenols can be detected in human urine, plasma, breast milk, amniotic fluid, placental tissue and umbilical cord blood in many countries (Chen et al., 2010; Schlumpf et al., 2010; Tan and Ali Mohd, 2003; Vandenberg et al., 2012; Vela-Soria et al., 2011; Woodruff et al., 2011).

There is a heightened awareness of the potential role of environmental factors in child development. During critical periods of development, including the prenatal period, exposure to a number of environmental chemical contaminants may adversely affect the growth, reproduction, and development in wildlife and humans, causing morphologic and functional alterations (Schonfelder et al., 2002). Many of these chemicals are known to impact the endocrine system of animals and humans. Several researches indicated that compounds with endocrine disrupting effects were associated with adverse birth outcomes (Govarts et al., 2012; Longnecker et al., 2001; Meeker et al., 2009). BPA, BP-3 and APs are all known as endocrine disrupting chemicals (EDCs) (Isidori et al., 2010; Li et al., 2012; Schlumpf et al., 2004), therefore, their effect on birth outcomes needs particular attention. So far, there is still limited...
evidence for adverse birth outcomes in association with phenol exposure in animal studies, and most of them mainly focus on BPA (Kim et al., 2001; Rubin et al., 2001; Savabieasfahani et al., 2006). Aside from BPA (Cantownwe et al., 2010; Miao et al., 2011; Padmanabhan et al., 2008), the epidemiologic studies regarding prenatal exposure to other phenols are also lacking. In addition, currently, little is known about the extent of exposure to these agents and their potential toxic effects on development in the general Chinese population.

Phenols are mainly excreted into urine (Ye et al., 2005). Therefore, total (conjugated and free) phenols measured in human urine can reflect individual internal exposure level (Ye et al., 2005) and has been widely used in exploring the relationships between exposure to those compounds and diseases as well as laboratory abnormalities (Chevrier et al., 2013; Kim and Park, in press; Lang et al., 2008).

Therefore, we investigated urinary levels of BPA, BP-3, 4-n-octylphenol (4-n-OP), 4-n-nonylphenol (4-n-NP) in pregnant women, and examined their relationship with birth outcomes, including birth weight, length and gestational age.

2. Material and methods

2.1. Study population

Pregnant women were recruited from hospitals affiliated to Nanjing Medical University between September 2010 and April 2012. Eligible women with singleton pregnancy were ≥18 years old, and they reported no assisted reproduction and medical complications (e.g., gestational or preexisting diabetes, hypertension, HIV infection or AIDS) (Wang et al., 2012). Newborn infants with severe neonatal illness [e.g., very premature births (delivery at <32 completed gestational weeks or birth weight <1500 g), genetic abnormalities or malformations] were further excluded (Wolff et al., 2008). A total of 592 women met the eligibility criteria. Of these, 567 women agreed to participate in this study (response rate: 95.8%). The study was approved by the Institutional Review Board of Nanjing Medical University. All participants provided written informed consent prior to the study.

2.2. Face-to-face interview

A questionnaire was conducted with each participant by face-to-face interview, collecting information as follows: demographic and socioeconomic information (maternal age, height, end-of-pregnancy weight, education level, household income, and address), maternal characteristics (dietary habits, any smoking or alcohol use during pregnancy), occupational history, exposures to environmental hazards and reproduction status. Maternal BMI in late pregnancy was calculated as end-of-pregnancy weight (kg) divided by the height (m) squared. Additionally, information about previous pregnancies, medications, medical conditions, complications during the current pregnancy and self-reported last menstrual period (LMP) was also obtained by interview and confirmed by medical records.

2.3. Measurement of fetal growth

Infant sex, birth date, parity, weight, and crown–heel length were obtained from hospital delivery logs and medical records. Date on clinical estimate of gestational age (ultrasound) was also collected. Gestational age was estimated based on the onset of LMP; if the LMP was unreliable or if there was a significant discordance between the clinical estimate and LMP (>2 weeks), the first clinical estimate of gestational age was used (Cheng et al., 2007; Eskenazi et al., 2004). Low birth weight was defined as <2500 g. Preterm delivery was defined as birth at less than 37 completed weeks of gestation.

2.4. Urinary phenols determination

Urine samples were collected from each subject during hospital admission for delivery, and were frozen at −20 °C until analysis. The total urinary concentrations of BPA, BP-3, 4-n-OP and 4-n-NP were measured with a sensitive method as previously described (Chen et al., 2012). Briefly, 4 ml of urine samples were incubated with beta-glucuronidase/sulfatase at 37 °C overnight, then the hydrolyzed compounds in the urine was purified by solid phase extraction. After dryness, the residue was redissolved in methanol. The solution was analyzed by ultra high performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS, Waters, USA). The limits of detection (LODs) for these chemicals were based on a signal to noise ratio (S/N) of three, LODs were 0.36 μg/L (BPA), 0.04 μg/L (BP-3), 0.02 μg/L (4-n-OP) and 0.02 μg/L (4-n-NP). Quality control (QC) samples which were prepared from spiked pooled urine were analyzed along with standards, blanks and unknown samples. Urinary creatinine (CR) concentrations were used to correct the metabolite concentrations for variable urine dilutions. CR concentrations were determined using an automated chemistry analyzer (7020 Hitachi, Tokyo, Japan).

2.5. Statistical analysis

Statistical analyses were performed using STATA statistical package (Version 9.2, Stata Corp, LP). Because of the high proportion of samples with target compounds that had levels below the detection limit, a three-level ordinal variable was formed: all samples with concentrations <LOD were assigned to the lowest group, and two equally sized groups were formed among the samples with detectable concentrations to form the middle- and high-exposure groups (Meeker et al., 2011).

Multivariate linear regression model was used to examine relationship between prenatal exposure to phenols and birth outcomes. Inclusion of covariates was based on biological and statistical considerations. Covariates were included in the final models if they showing associations (p < 0.1) with birth outcomes or biomarkers by univariate analysis (Wolff et al., 2008). We included the set of covariates in models as follows: urinary CR, gestational age at birth (not included in models predicting gestational age), maternal age, body mass index (BMI) in late pregnancy and parity. Urinary CR was included as continuous variables to adjust for urinary dilution (Meeker et al., 2011). Maternal age, gestational age and BMI in late pregnancy were also modeled as continuous variables, whereas parity (0, or ≥1) were treated as a binary variable. Smoking and alcohol were not included in the final models because very few women reported use. Considering that the association between exposure and birth outcomes might differ by infant sex, we also performed stratified analysis for boys and girls. Tests for trend were performed for ordinal categories of target compounds in the adjusted regression models with integer values (0, 1, 2) (Meeker et al., 2011); a p-value of <0.05 indicated statistical significance.

3. Results

3.1. Main demographic characteristics of study participants

Maternal and infant characteristics in this study were presented in Table 1. Maternal age ranged from 18 to 45 years of age with mean (SD) of 27.8 (4.7). Average (SD) maternal BMI in late pregnancy was 27.6 (4.4) kg/m². Approximately three-fourths of the women were primiparous and over half (58.9%) had graduated from high school or college. Very few mothers reported tobacco smoking (4 mothers) and regular alcohol use (9 mothers) during pregnancy. For the newborns, 50.6% of them were male. The mean (SD) birth weight, length and gestational age were 3370 (556) g, 50.2 (2.4) cm, and 39.1 (1.9) weeks, respectively. A total of 5.7% newborns with missing data, c Newborns with missing data, d a Values are end-of-pregnancy weight (kg) divided by the height (m) squared.

Table 1

| Characteristics of mothers and newborns in this study (n = 567). |
|-----------------|-----------------|
| Maternal characteristics |
| Maternal age (year, mean ± SD) | 27.8 ± 4.7 |
| Parity (%) |
| 0 | 73.1 |
| ≥1 | 26.9 |
| Education (years, %) |
| ≥9 | 41.9 |
| 10–12 | 22.2 |
| ≥13 | 35.9 |
| Maternal height (cm, mean ± SD) | 161.7 ± 4.3 |
| End-of-pregnancy weight (kg, mean ± SD) | 72.2 ± 11.8 |
| BMI in late pregnancy (kg/m², mean ± SD)a | 27.6 ± 4.4 |
| Smoking during pregnancy (%) | 0.7 |
| Drinking during pregnancy (%) | 1.6 |
| Newborn characteristics |
| Sex (%) |
| Male | 50.6 |
| Female | 49.4 |
| Birth weight (g, mean ± SD)b | 3370 ± 556 |
| Body length (cm, mean ± SD)b | 50.2 ± 2.4 |
| Length of gestation (week, mean ± SD)d | 39.1 ± 1.9 |

a Values are end-of-pregnancy weight (kg) divided by the height (m) squared.
b Newborns with missing data, n = 3.
c Newborns with missing data, n = 10.
d Newborns with missing data, n = 16.
(n = 32) of infants were born of low birth weight, and 4.9% (n = 27) were preterm births.

3.2. Maternal urine phenols exposure levels

Table 2 showed urinary concentrations of phenols in the present subjects along with the detection limit for those compounds. Among those compounds, BPA and BP-3 exhibited relatively higher detection frequencies. BPA showed highest urinary levels, followed by BP-3, 4-n-OP and 4-n-NP.

3.3. Relationship between prenatal phenols exposure and birth outcomes

Crude and adjusted regression coefficients (β) and 95% confidence intervals (CI), based on the entire sample, for the relationship between phenol exposure levels and birth outcomes are presented in Table 3. The crude and adjusted results were similar. Categories of urinary BP-3 concentration were associated with a decrease in gestational age (p-value for trend = 0.03). Between middle and low exposure groups, we also found that BPA was negatively associated with gestational duration (βadjusted = −0.48 week; 95% CI: −0.91 to −0.05). No significant relationships were observed between exposure to APs and any of birth outcomes.

In Fig. 1, Table S1 and S2 (Supplementary material), results of stratification analyses performed for boys and girls revealed possible sex-specific association of prenatal exposure to BP-3 and BPA with gestational age. We found that, in infant boys, BP-3 exposure categories were related to decreased length of gestation [p-value for trend = 0.006; Fig. 1], and BPA showed significantly negative relationship with gestational duration between middle and low exposure groups [βAdjusted = −0.78 week; 95% CI: −1.44 to −0.11; Table S1 and Fig. 1]. Results in boys were consistent with those in all infants, while we did not observe significant association for girls.

4. Discussion

As shown in many studies, a number of chemicals, such as PCBs, organochlorine pesticides and phthalates can pass through the placenta (Correia Carreira et al., 2011; Mose et al., 2007; Waliszewski et al., 2000) and prenatal exposure to them have been demonstrated to associate with decreased gestational duration (Meeker, 2012; Meeker et al., 2009; Wigle et al., 2008). Among phenols, BPA has been confirmed to easily cross the blood–placenta barrier at low environmentally relevant levels (Balakrishnan et al., 2010; Schonfelder et al., 2002; Wan et al., 2010) and BP-3, with a similar chemical structure to BPA, may also pass the blood–placenta barrier (Krause et al., 2012). Additionally, the rate of clearance of phenols, such as BPA, is slower in the fetus than in maternal blood (Takahashi and Oishi, 2000), because most urinediphosphate–glucuronosyltransferase (UDPGT) isoenzymes which involved in biotransformation and elimination of a wide variety of xenobiotic phenols did not appear until after birth (Coughtrie et al., 1988). Thus, the fetus is especially vulnerable to these phenols and prenatal exposure to them might adversely affect fetal growth and gestational duration.

Among numerous chemicals, EDCs are especially of concern because once inside the body, they can affect the endocrine and
reproductive system along multiple points including the hypothalamus and the gonad (Robins et al., 2011). Thus, the maternal—placental—fetal unit, which is now accepted to represent the interaction of three endocrine systems changing throughout pregnancy in mammals (Bigsby et al., 1999), can also be the target of the EDCs. Indeed, prenatal exposures to EDCs, such as PCBs, organochlorine pesticides and phthalates, have been indicated to be related to shortened length of gestation in many epidemiological studies (Meeker, 2012; Meeker et al., 2009; Wigle et al., 2008). Both BP-3 and BPA exhibited estrogenic activities and were possessed of binding affinity to estrogen receptor $\alpha$ (ER$\alpha$), estrogen receptor $\beta$ (ER$\beta$) and some other steroid receptor, such as estrogen receptor-related receptors (ERRs) (Gomez et al., 2005; Lee et al., 2012; Li et al., 2012; Matsushima et al., 2007; Schlecht et al., 2004; Schreurs et al., 2005). Evidence for decreased gestational duration in relation to BPA has also been reported (Cantonwine et al., 2010). Recently, a proposed molecular biology of endocrine disruption lead to adverse birth outcomes was that, the placenta is central to successful pregnant outcomes and the estrogen-related receptor gamma (ERR$\gamma$) protein, exhibited a strong affinity for BPA, has been described as highly expression in the placenta (Poidatz et al., 2012), so that EDCs like BPA may exert its adverse influence on pregnant outcomes through binding and activation of this receptor and its downstream signaling (Cantonwine et al., 2010). Therefore, our results that shortened gestational age was associated with the categories of BP-3 exposure and with BPA concentration between middle and low exposure group (Table 3) may be consisted with their endocrine disrupting activities. Our data provides a new evidence of potential developmental risk of prenatal exposure to phenols on gestational duration.

In the present study, stratified analysis for infant sex revealed the sex-specific association of BP-3 and BPA exposure with gestational age. The phenomenon of sex-specific association was also present in many other researches regarding effects of exposure to environmental chemical contaminants on birth outcomes (Chou et al., 2011; Philippat et al., 2012; Wang et al., 2012; Wolff et al., 2008). However, these associations still require further confirmation.

APs, which are also the commonly mentioned phenolic endocrine disruptors (Bonefeld-Jorgensen et al., 2007; Chen et al., 2010), showed no association with any of birth outcomes in this study. It may occur because the detection rate of 4-n-OP and 4-n-NP were too low to examine the statistical associations and their relatively lower exposure levels may not elicit biological effects.

To the best of our knowledge, we present the first human study in China to examine the relationship between prenatal exposures to various phenols and birth outcomes. However, as in many studies (Philippat et al., 2012; Wang et al., 2012; Wolff et al., 2008), we measured urinary concentrations of these compounds at a single time point exclusively. We are limited in our ability to understand the average cumulative dose from different sources and to what extent these measurements accurately reflected the exposure throughout the entire critical period of fetal growth. Future research should increase the number of urine samples collected during the whole gestation and repeatedly measure those samples to provide a more accurate estimate of the average exposure.

5. Conclusions

In summary, we investigated the relationship of prenatal exposure to phenols with fetal growth and gestational duration in China. Prenatal BP-3 and BPA exposures were found to be associated with shortened gestational duration. Further study may be needed to confirm these results and identify potential mechanisms.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2013.03.023.

References


Takahashi, O., Iorsha, S., 2008. Inhibition of expression of orally administered 2,2-Bis(4-hydroxyphenyl) propane (Bisphenol A) in pregnant rats and the placental transfer to fetuses. Environ. Health Perspect. 108, 931–935.


