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Prenatal exposure to phthalate esters and PAHs and birth outcomes

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ABSTRACT

The purpose of this study was to investigate the relationship between the levels of prenatal exposure to phthalate ester and PAHs and birth outcomes among 149 Japanese pregnant women.
Urinary concentrations of 9 phthalate ester metabolites, mono methyl phthalate (MMP), mono ethyl phthalate (MEP), mono-n-butyl phthalate (MnBP), mono benzyl phthalate (MBzP), mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), mono-iso-nonyl phthalate (MINP) and mono-n-octyl phthalate (MnOP) and PAHs metabolite (1-hydroxypyrene, 1-OHP) were analyzed in spot urine samples collected from pregnant women. Correlation analysis and multiple regression analysis were conducted between the concentrations of maternal urinary metabolites and birth outcomes such as birth weight, birth length, head circumference and gestational age. Creatinine-corrected concentration (geometric mean; μg/g cre) was 9.14 (MMP), 9.76 (MEP), 51.6 (MnBP), 5.62 (MBzP), 5.45 (MEHP), 10.6 (MEHHP), 11.3 (MEOHP), 0.031 (MINP), 0.025 (MnOP) and 0.121 (1-OHP). These concentrations are comparable with literature value. The relationships between prenatal exposure to phthalate esters and birth outcomes were not significant. Statistically significant negative correlation was observed between 1-OHP and birth weight, birth length and head circumstances although the correlation was insignificant when only non-smokers were included in multiple regression analysis. In conclusion, we found that prenatal exposure to phthalate esters or PAHs did not affect birth outcomes at the exposure level of the present subjects.

1. Introduction

Children are vulnerable to exogenous factors including hazardous chemicals because various tissues and organs are developing from prenatal to adolescence (IPCS, 2006). During fetal period, there is "critical window" for the development of nervous, reproductive, endocrine, cardiovascular, immune and respiratory systems when even a subtle exposure to chemicals may adversely affect morphologies and functions of the systems. Therefore it is critically important to examine prenatal chemical exposure and its effect on human health.

Birth outcome, such as birth weight, has been routinely used for the evaluation of fetal development in experimental animal studies. Moreover, human birth outcome has been indicated to predict occurrence of diseases, such as cardiovascular disease, in the later life (Lau and Rogers, 2004). It is also indicated that negative relationships were present between birth weight and IQ score in childhood and in adulthood even in the normal birth weight range (Shenkin et al., 2004).

In this study, the relationships are evaluated between birth outcomes and the exposure to the ubiquitous organic pollutants in our environment, i.e., phthalate esters and polycyclic aromatic hydrocarbons (PAHs).

Phthalate esters are frequently used as plasticizers and solvents and thus are abundantly present in our environment. There are a number of *in vitro* and *in vivo* developmental and reproductive studies of this group of compounds (Lyche et al., 2009). In animal studies, birth weight was decreased at the administered dose of 509–794 mg/kg bw/day among rat offspring followed by prenatal exposure to Di-n-butyl phthalate (Wine et al., 1997). Several epidemiological studies have been published on the effect of prenatal phthalate exposure on birth outcomes (Latini et al., 2003; Wolff et al., 2008; Zhang et al., 2009; Adibi et al., 2009). In some studies, statistically significantly negative correlation has been observed between birth outcomes and phthalate exposure level though this relationship was not consistent across the studies. These have raised concerns about adverse effects of prenatal exposure to phthalate esters in humans. The authors previously showed that Japanese pregnant women were exposed to various phthalate esters in daily life (Suzuki et al., 2009). Therefore the purpose of the present study was to examine prenatal phthalate exposure and birth outcomes in Japanese population.

Polycyclic aromatic hydrocarbons are another group of compounds ubiquitously present in our environment. Some of the PAHs are well known carcinogen but their developmental effect has recently been highlighted. Perera et al. (2003) found significantly

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negative correlation between maternal exposure level of atmospheric PAHs and birth sizes in urban population. On the other hand, as a part of our recent study, we have examined the relationships between birth outcomes and PAH exposure using urinary metabolite (1-hydroxypyrene, 1-OHP) as biomarker among 50 Japanese pregnant women and did not find significant relationship (Niwa et al., unpublished). An additional purpose of the present study was to re-evaluate the relationship using larger sample size.

2. Subjects and methods

2.1. Subjects

One hundred and forty-nine Japanese pregnant women without apparent clinical symptoms agreed to participate in this study after being explained the purposes of the study in the Department of Obstetrics and Gynecology of a hospital in Tokyo during 2005–2008. Ethical committees of the hospital and the University of Tokyo approved this study.

2.2. Sampling

Spot urine sample was obtained from the subject at 9th to 40th of gestational weeks (mean ± SD: 29 ± 8). The urine samples were stored at −20 °C until chemical analyses. Target metabolites were not detected in any procedural blanks indicating that there was no contamination from containers and other devices.

2.3. Analytical methods for phthalate ester metabolites and PAH metabolite

Urinary concentrations of 9 phthalate ester metabolites, mono methyl phthalate (MMP), mono ethyl phthalate (MEP), mono-n-butyl phthalate (MnBP), mono benzyl phthalate (MBzP), mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), mono-iso-nonyl phthalate (MINP) and mono-n-octyl phthalate (MnOP) and PAHs metabolite (1-OHP) were measured in this study. The detailed analytical methods were described in Suzuki et al. (2009) and in Chetiyanukornkul et al. (2002) for phthalate ester metabolites and 1-OHP, respectively. Briefly, for phthalate ester metabolites, 1 ml of urine sample was incubated with beta-glucuronidase at 37 °C for 60 min. After solid phase extraction, the eluate was dried under nitrogen gas stream and the residue was re-dissolved in ultra pure water. The solution was analyzed by HPLC tandem mass spectrometry (HPLC-MS/MS, Agilent, California, USA for HPLC and Micromass Quattro Ultima, Manchester, UK for tandem MS, respectively.). For 1-OHP, 1 ml of urine sample was treated with beta-glucuronidase/arylsulfatase, and the hydrolyzed 1-OHP in the urine was purified by solid phase extraction. The concentration of 1-OHP was measured by high-performance liquid chromatography with fluorescence detector (HPLC-FL, LaChrom Elite model HPLC system consisting of L-2130 model pump and L-2480 model fluorescence detector, Hitachi High-Technologies Co. Ltd., Tokyo, Japan).

Internal quality control of the urinary metabolite analyses was carried out by periodical blank measurement, recovery test and the analysis of intra-laboratory reference urine samples. Moreover, our laboratory took part in Intercomparison Programme 40 of the German External Quality Assessment Scheme in 2007 for some phthalate ester metabolites and 1-OHP in urine.

2.4. Anthropometric measurement and maternal characteristics

Birth weight, birth length, and head circumstances of newborns were measured by clinical nurse at delivery with the standard measurement procedures. Information on maternal weight, height, age and smoking status during pregnancy was obtained from medical record. The maternal profile is shown in Table 1. Mean maternal age and BMI were 31.9 and 21.3, respectively. Of the 149 mothers, 15 (10%) smoked during pregnancy.

2.5. Statistical analyses

All statistical analyses were performed with SPSS for Windows ver. 12.0 (SPSS Japan inc., Tokyo Japan). Urinary metabolite concentration for phthalate esters and PAHs was log-transformed for the following parametric statistical analyses: multiple regression analysis for the evaluation of relationships between outcomes and urinary metabolite levels, unpaired *t*-test for the comparison of urinary metabolite levels between low-birth-weight (<2500 g) and normal weight newborns, and one-way ANOVA was performed for the category analysis of quartile of urinary metabolites concentration. Half of the detection limit value was substituted for non-detectable sample for statistical analysis.

3. Results

3.1. Birth outcomes of 149 newborns

The results of anthropometric measurements of 149 newborns born to the present subjects are shown in Table 1. There were 80 males and 69 females and no significant difference was observed for birth weight, birth length, head circumstances and gestational age between sexes. The rate of low-birth-weight (LBW) newborns was 4% and it was lower than the national average in Japan (8.5% and 10.6% of male and female newborns, respectively). There were two preterm infants in the 149 subjects. The preterm birth rate of 1.5% in this study was lower than the national average 5.8% in 2008 (Japanese Ministry of Health, Labor and Welfare, 2010).

3.2. Urinary concentrations of phthalate ester metabolites and 1-OHP of the subjects

Table 2 shows urinary concentrations of 9 phthalate ester metabolites and 1-OHP of the present subjects along with the detection limit for those metabolites. In this table, uncorrected concentrations and creatinine-corrected concentrations were given. Number of samples for 1-OHP was 128 because of limited sample volume. Eight of the 10 urinary metabolites were detected in nearly 100% of the subjects while the detection rate of MINP and MnOP was relatively low (7 and 10%, respectively). Therefore MINP and MnOP were not included in further statistical analysis. The distributions of the concentrations in urine skewed towards higher value and they are assumed to be log-normal distribution: log-transformed value was used in the following statistical analyses. The geometric mean uncorrected concentrations of MMP, MEP, MnBP, MBzP, MEHP, MEHHP, MEOHP, MINP, MnOP and 1-OHP were 6.95, 7.42, 46.2, 4.27, 4.14, 8.08, 8.60, 0.024, 0.019 and 0.085 ng/ml, respectively. Note that one half of detection limit value was substituted for non-detectable samples for the calculation of the mean. Creatinine-corrected concentrations (geometric mean; μg/g cre) were 9.14, 9.76, 51.6, 5.62, 5.45, 10.6, 11.3, 0.031, 0.025, and 0.121 for MMP, MEP, MnBP, MBzP, MEHP, MEHHP, MEOHP, MINP, MnOP and 1-OHP, respectively. Because there were no statistical differences in urinary concentrations of phthalate esters and PAHs metabolites between the subjects who delivered LBW and normal weight

Table 1
Profile of the149 mothers and 149 newborns in this study.

	Mean	SD
Maternal age [year]	31.9	4.5
Pre-pregnancy maternal weight [kg]	53.8	8.9
Maternal height [cm]	158.9	5.2
Pre-pregnancy maternal BMI	21.3	3.2
Number of smoking mother during pregnancy[a]	15 (10%)	
Number of non-smoking mother during pregnancy[a]	134 (90%)	
Birth weight of newborns [g]	3098	395
Birth length of newborns [cm]	48.6	2
Birth head circumstances of newborns [cm]	33.4	1.3
Gestational age [weeks]	39.6	1.2
Number of low birth weight newborns	6 (4%)	
Number of preterm births [<37]	2	
Number of term births [37–42]	147	
Birth order [number]		
1	59	
2	57	
3	27	
>3	6	

[a] Self reported.