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Urinary paraben concentrations among pregnant women and their matching newborn infants of Korea, and the association with oxidative stress biomarkers

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HIGHLIGHTS

- Four parabens were measured in the urine of pregnant women and their matching infants.
- Urinary EP levels of pregnant women were 4–9 folds higher than other countries.
- MP or EP levels were associated with stress markers in maternal or fetal urine.
- Consequences of paraben exposure among sensitive humans deserve further study.

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ABSTRACT

Parabens have been used in multiple products including personal care products, pharmaceuticals, and foods for more than 50 years but increasing numbers of studies have raised concerns on their safety. The present study was designed to determine urinary paraben levels among pregnant women and their matching newborn infants (<48 h after delivery), and the association between paraben levels and stress markers. Pregnant women (n = 46) and their matching newborn infants were recruited from four university hospitals located in Seoul, Ansan and Jeju of Korea, 2011. Parabens including methyl paraben (MP), ethyl paraben (EP), n-propyl paraben (PP), and n-butyl paraben (BP) were measured in the urine using an automatic, high throughput online SPE–LC–MS/MS method. Urinary concentrations were normalized with specific gravity (SG). Free cortisol, malondealdehyde (MDA) and 8-hydroxydeoxyguanosine (8-OHdG) were measured in the urine as stress marker. Urinary MP was detected as the highest, and BP was detected as the lowest paraben in the urine samples of both pregnant women and their infants. Significant correlations between paraben concentrations of maternal and their newborn infant's urine were observed. The levels of urinary parabens among Korean pregnant women are comparable to those reported elsewhere, except for EP which were 4–9 folds higher than pregnant women of other countries. The ratios of infant to maternal urinary paraben concentrations varied between 0.5 and 0.6 for MP and PP, but approximately 10 fold lower for EP. Urinary MP or EP levels were associated with several oxidative stress related biomarkers such as urinary 8-OHdG and MDA, even after the adjustment of relevant covariates such as maternal age, mode of delivery, pre-pregnancy BMI, gestational age and parity. This is the first study that reported the levels of major parabens in the first urine of newborn infants. Further studies are warranted to understand the implications of paraben exposure among biologically susceptible human populations.

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1. Introduction

Parabens are esters of *p*-hydroxybenzoic acid, having antifungal and antibacterial properties. For this reason, these compounds have been used in multiple products including personal care products, pharmaceuticals, and foods (Andersen, 2008). Antimicrobial activity of parabens is thought to increase with the length of alkyl group, e.g., from methyl to n-butyl (Han and Washington, 2005). The octanol/water partition coefficients of parabens increase as the carbon number of the alkyl chain of parabens increases. The logKow value for methyl paraben (MP) is 1.66, for ethyl paraben (EP), 2.19, for propyl paraben (PP), 2.71, and for butyl paraben (BP), 3.24.

Recently parabens have been detected in high levels in humans (Calafat et al., 2010; Meeker et al., 2011). Since parabens and their metabolites can be measured in urine, urinary levels of parent parabens may be used as biomarkers of recent human exposure (Ye et al., 2006a). Exposure may occur through ingestion, inhalation, or dermal absorption. Upon absorption, parabens are rapidly metabolized mainly into *p*-hydroxybenzoic acid and its respective glucuronic and sulfuric acid conjugates. Parent parabens in their free or conjugated form can be excreted in the urine following the skin application. Urinary paraben concentrations were reported to correlate to those levels in both serum and seminal plasma in 60 healthy Danish men (Frederiksen et al., 2011).

Parabens have been frequently detected in pregnant women worldwide, with MP being the greatest concentrations, followed by PP, EP, and BP. Among 120 Spanish pregnant women, the average level of MP, PP, EP, and BP was 191, 29.8, 8.8, and 2.4 μg/L, respectively. In their children's urine, the average level of MP, PP, EP, and BP was 150, 21.5, 8.1, and 1.2 μg/L, respectively, suggesting similar sources of exposure (Casas et al., 2011). Similar patterns of paraben concentrations were reported among pregnant women in Japan and USA (Shirai et al., 2012; Smith et al., 2012). While urinary paraben concentrations are quite variable in women during pregnancy, a single urine measurement during pregnancy could represent gestational exposure (Smith et al., 2012). Paraben exposure during pregnancy is important because prenatal exposure to parabens may affect the health of infants. To our knowledge, the levels of parabens in the newborn infant's urine have never been reported.

Although parabens have been used for more than 50 years and are generally considered as safe, several studies have raised concerns on the safety of parabens (Prusakiewicz et al., 2007; Tavares et al., 2009). Exposure to parabens may modulate or disrupt the endocrine system and cause oxidative stress, which may cause harmful consequences in animals and humans (Darbre and Harvey, 2008; McGrath, 2003). In pregnant rats, exposure to BP caused adverse effects on the reproductive organs of the male offspring (Kang et al., 2002). BP can cause oxidative stress by inhibiting anti-oxidants, and was reported to increase malondialdehyde (MDA) in mouse liver (Shah and Verma, 2011). MP and EP may also induce oxidative stress by mediating erythrocyte glutathione (GSH) conjugates of hydroquinone by reacting with $^1O_2$ and GSH (Nishizawa et al., 2006). PP led to an increase of DNA damage in Vero cell (Martin et al., 2010). Oxidative stresses caused by parabens were also reported in other studies (Arikan et al., 2006; Nishizawa et al., 2006), but such links in humans have seldom been reported.

The present study was designed to determine the urinary levels of parabens in pregnant women and their matching infants in Korea. The association between urinary paraben levels and the biomarkers of oxidative stresses was also evaluated. The results of this study will help understand the levels of paraben exposure among sensitive human populations, and develop management options on these compounds.

2. Materials and methods

2.1. Study population and sample collection

Pregnant women (n = 46) and their matching newborn infants (n = 46) were recruited from four university hospitals located in Seoul, Ansan and Jeju of Korea between February and December, 2011 (Table 1). Urine samples were collected from pregnant women within a day before delivery (except for one subject from whom urine was sampled a day after the delivery) and from the infants within 48 h after birth. For collection of the infant urine, urine collection bag was used. Urine collection bag has an adhesive inlet which can be attached around external genitalia of the infants. After the collection, the urine samples were moved to conical tubes. Urine samples were then stored at −80 °C immediately, until analysis. In addition, placenta tissue (~1 × 1 × 1 cm) was sampled immediately following birth, and was stored in −80 °C freezer. Placenta sample was taken approximately 1–1.5 cm below the fetal membrane to avoid membrane contamination. A paraben-containing body-wash product was used in one participating hospital for the newborn infants (n = 9).

One-on-one interviews with participating pregnant women were conducted, and demographic parameters, physiological data, and pregnancy history at the time of enrollment were asked. Medical records regarding current or previous health status and gestational period were abstracted. Institutional Review Boards of School of Public Health, Seoul National University, and all participating university hospitals approved the study. Informed consents were obtained from the participating women. All samples and data were processed blind.

2.2. Chemicals and analysis

Four parabens including MP, EP, PP, and BP were measured in the urine. Target chemicals and internal standards, i.e., MP-d_4, EP-d_4, PP-d_4, BP-d_4 were purchased from Sigma-Aldrich (Yongin, Korea), and CDN ISOTOPE (Quebec, Canada), respectively. Distilled water and solution were bought from Burdick & Jackson (Morristown, NJ, USA) and ammonium formate, ammonium acetate and formic acid were obtained from Fluka (Buchs, Switzerland).

Urine sample was prepared by enzyme hydrolysis and analyzed using online SPE–LC–MS/MS method, following Ye et al. (2006a, 2006b) with minor modifications. A 100 μL aliquot of the urine was mixed with 830 μL of 0.1 M acetic acid, 10 μL enzyme solution (β-glucuronidase/sulfatase), and 10 μL of internal standard solution (200 ng/mL). After incubating at 37 °C for 4 h, and subsequent centrifuging at 2500 rpm for 10 min, the supernatant was transferred to glass vial and analyzed by online SPE–LC–MS/MS system. Calibration curve was derived from artificial urine spiked with standard (0.1. 0.5, 1, 2, 5, 10, 50, 100, 250, or 500 ng/mL). Artificial urine was prepared in D.W. (100 mL) with potassium chloride (0.76 g), sodium chloride (1.7 g), urea (4.9 g), citric acid (0.206 g), ascorbic acid (0.068 g), potassium phosphate (0.236 g), creatinine (0.28 g), sodium hydroxide (0.128 g), sodium bicarbonate (0.094 g), and sulfuric acid (0.056 ml) following Gustafsson and Uzqueda (1978).

Nanopace II (Shiseido, Tokyo, Japan), equipped with autosampler, dual pump, column oven, vacuum degaser and switching valve, was

Table 1
Characteristics of pregnant women and their newborn infants.

Variable	n	Range	Mean	SD	Median
Pregnant women (n = 46)					
Age (year)	46	22–39	33	3.54	33
BMI (kg/m^2)	34	13.80–27.80	21.08	3.25	20.75
Parity	46	0: 19, ≥1: 27			
Gestational age at delivery (days)	46	256–288	272.02	9.01	274
Newborn infants (n = 46)					
Sex		Male: 25, Female: 21			
Birth weight (kg)	46	2.47–4.15	3.28	0.34	3.27
Birth height (cm)	38	33.50–54.00	49.67	3.23	49.80
Birth circumference (cm)	37	31–49	34	2.83	34