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Polycyclic aromatic hydrocarbon (1-OHPG and 2-naphthol) and oxidative stress (malondialdehyde) biomarkers in urine among Korean adults and children

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ABSTRACT

Using the urinary biomarkers 1-hydroxypyrene-glucuronide (1-OHPG), 2-naphthol, and malondialdehyde (MDA), we evaluated seasonal and regional variations in polycyclic aromatic hydrocarbon (PAH) exposure and oxidative stress among Korean adults and children. In total, 322 children (175 male and 147 female) and 332 adults (47 male and 285 female) were recruited in two regions of Korea, one representing a metropolitan area (Seoul/Incheon) and the other an industrial (Pohang) area, from winter 2002 to spring 2003. The subjects voluntarily gathered their first morning urine void, which was immediately transported to our laboratory and stored at −20 °C. Urinary 1-OHPG was measured by synchronous fluorescence spectroscopy, 2-naphthol by HPLC, and urinary MDA by HPLC with a UV detector. The median urinary 1-OHPG concentration tended to be higher in the industrial region than in the metropolitan region (0.92 *vs.* 0.77 ng/mL; $p = 0.03$), and higher in winter than in spring (0.95 *vs.* 0.73 ng/mL; $p < 0.001$). The median 2-naphthol concentration was also higher in the industrial region than in the metropolitan region (21.0 *vs.* 12.3 ng/mL; $p < 0.0001$), but was higher in spring than in winter (19.7 *vs.* 10.3 ng/mL; $p < 0.0001$). The median MDA concentration was significantly higher in winter than in spring (2.19 *vs.* 1.03 μmol/L; $p < 0.0001$), whereas regional variation in MDA was observed only in female adults ($p = 0.02$). In winter, the level of 1-OHPG was higher in children exposed to environmental tobacco smoke than in unexposed children (0.94 *vs.* 0.86 ng/mL; $p = 0.02$). Our results indicate that both region and season can significantly influence the levels of PAH exposure and oxidative stress biomarkers.

Introduction

Adverse health effects of exposure to polycyclic aromatic hydrocarbons (PAHs), which are the result of incomplete burning of organic substances in occupational setting or in the environment, have been frequently reported to include asthma, allergies, rhinitis, and other respiratory diseases. Benzo[*a*]pyrene, a common component of PAHs, and several other PAH components from various exposure sources, such as tobacco smoking, combustion of coal, and vehicle exhaust, are classified as Group 1 carcinogens (*carcinogenic to humans*) by the IARC (International Agency for Research on Cancer). Especially among children, PAH exposure can result in congenital defects in the neural tube (Naufal et al., 2010) and affect neurodevelopment (Perera et al., 2008). While there are various kinds of biomarkers that could reflect levels of PAH exposure, urinary 1-hydroxypyrene-glucuronide (1-OHPG) has been suggested as a sensitive exposure biomarker for low level PAH exposures and as a more suitable internal dose biomarker than 1-OHP (Strickland and Kang, 1999). The level of 2-naphthol, a metabolite of naphthalene, in urine was reported to reflect more specifically the exposure to PAHs in ambient air compared to that based on 1-OHP levels (Kang et al., 2002; Kim et al., 2001). Exposure to PAHs is known as one cause of reactive oxygen species (ROS) induction and, as a result, lipid peroxidation or DNA damage (Hussain et al., 2003; Kelly, 2003). Although a number of biomarkers can be used to measure oxidative stress, malondialdehyde (MDA) has been suggested to be a highly sensitive indicator, in terms of

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consistency and stability, of low-level exposure to PAHs (Kang et al., 2005).

The levels of PAH-related biomarkers have been shown to be influenced by the PAH source, such as air pollution, cooking/heating fuel, smoking and environmental tobacco smoke (ETS) exposure, and dietary habits. Several studies have investigated the relationship between PAH exposure and levels of selected biomarkers. However, a limited number of studies have evaluated regional and seasonal effects. PAH exposure biomarker levels have been reported to be influenced by regional factors, including a region's industrial conditions (Gilbert and Viau, 1997; Lee et al., 2007b, 2009b; Mucha et al., 2006) or its traffic conditions (Fiala et al., 2001; Hansen et al., 2006; Yang et al., 2003). In addition, seasonal variation of biomarker levels is another interesting research topic that has been investigated by several authors (Lee et al., 2009b; Pastorelli et al., 1999; Perico et al., 2001).In the present study, we measured and compared PAH exposure biomarkers among Korean populations in different regions and seasons in order to characterize PAH exposure profiles for adults and children, after adjustment for environmental tobacco smoke.

Methods

Study subjects and questionnaire survey

This study was carried out in two regions in Korea, a metropolitan region (Seoul/Incheon) and an industrial region that contains the largest steel mill company in Korea (Pohang in southeast Korea). This study recruited students and their parents in school. We note that adult subjects were one or both the parents of the children and mothers were more willing to participate in our study than fathers. Response rates of children recorded Seoul (70/175, 40%), Incheon (127/174, 73%) and Pohang (125/252, 50%). A total of 322 children (175 male and 147 female) and 332 adults (47 male and 285 female) were recruited in winter 2002 and spring 2003. Height and weight of study subjects were measured on site. A structured questionnaire was used to collect information on smoking status [current smoker (>100 cigarettes/lifetime) *vs.* ex- or non-smoker], ETS exposure [exposed (at least one smoker in the family) *vs.* unexposed], heating fuel used (gas, central heating system, oil, wood, or coal), type of residential area (agricultural, residential, business, or industrial), and consumption of fried, broiled, or barbecued meat and fish. Frequency of food consumption was categorized into '>2/day', '1/day', '1/2 days', '1/3 days', '1/week', '1/2 weeks', '1/month' and 'none'.

Urine sampling procedure

Urine samples were collected for the analysis of PAH metabolites and oxidative stress biomarkers. A 500 mL urine bag and informed consent form were given to each participant and asked to collect the first morning urine void in the urine bag next day and bring it to his/her school. Collected urine samples were immediately transported to laboratory and stored in 15 mL cornical tube until analysis at −20 °C.

Measurement of PAH metabolites and an oxidative stress biomarker in urine

1-Hydroxypyrene glucuronide (1-OHPG)

A 4 mL aliquot of each urine sample was treated with 1 N HCl at 90 °C for 1 h to hydrolyze acid-labile conjugated metabolites. Urinary 1-OHPG was stable after acid hydrolysis and was loaded on methanol/water primed Sep-Pak C_{18} cartridges (Waters, Milford, MA, USA). Samples were washed with the same volume of water and 30% methanol in water, and eluted by the same volume of 80% methanol in water. The eluted samples were concentrated to around 1/8th of the initial volume under vacuum with mild heating, and the volume was adjusted up to 4 mL with 7.5 mM phosphate-buffered saline (PBS) (pH 7.4). The samples were loaded on the immuno-affinity columns, which consisted of CNBr-activated Sepharose 4B coupled with monoclonal antibody 8E11 which recognizes several PAH-DNA adducts and metabolites, including 1-OHPG. This was followed by washing with 4 mL of 7.5 mM PBS and 25% methanol in 7.5 mM PBS. Urinary 1-OHPG fractions were eluted with 70% methanol in 7.5 mM PBS and subsequently dried. After re-dissolving in 2 mL of water, the samples were measured by synchronous fluorescence spectroscopy (Perkin Elmer LS50B Luminescence spectrometer, Norwalk, CT, USA) with wavelength difference 34 nm of excitation–emission (Strickland et al., 1994). The limit of detection (LOD) was 0.01 ng 1-OHPG/mL urine. The recovery of the assay was 82% and the coefficient of variation was 9% (Lee et al., 2009a,b).

2-Naphthol

Urinary 2-naphthol levels were determined using reverse phase high performance liquid chromatography (HPLC). Briefly, a 0.5 mL urine sample was buffered with 50 μL of 2.0 mol/L sodium acetate buffer (pH 5.0) and hydrolyzed with 10 μL β-glucuronidase/sulfatase. The mixture was incubated at 37 °C for 16 h in a shaking water bath. After hydrolysis, 0.5 mL acetonitrile was added to the mixture. The mixture was centrifuged and 100 μL of the supernatant taken for HPLC application. The HPLC system consisted of a Young-Lin SP930D HPLC pump, a Young-Lin automated gradient controller, a MIDAS 830 autosampler, and a JASCO FP-2020 Plus fluorescence detector. The following HPLC parameters were used: column, Sunfire C_{18} (4.5 mm × 250 mm); mobile phase, 50% acetonitrile in water; and flow rate, 0.8 mL/min. The excitation/emission wavelengths used in the detection of 2-naphthol were 227/355 nm. The limit of detection was 0.5 ng/mL, and the coefficient of variation was <15% (Lee and Kang, 2008).

Malondialdehyde

A common method of measuring MDA is based on its reaction with 2-thiobarbituric acid. A 10 mmol/L stock standard of MDA was prepared by dissolving 123.5 μL 1,1,3,3-tetraethoxypropane in 50 mL ethanol (40% ethanol by volume). Adducts of 2-thiobarbituric acid-MDA were prepared in glass tubes with a polypropylene stopper. In each tube, 300 μL phosphoric acid (0.5 mol/L) was mixed with 50 μL urine and 150 μL 2-thiobarbituric acid reagent. The mixtures were incubated at 95 °C for 1 h, and methanol (500 μL) was added to each tube. After 5 min centrifugation (5000 × *g*), the samples were analyzed using HPLC on a 4.6 mm × 150 mm Sunfire C_{18} column with a 532 nm wavelength UV detector. The mobile phase comprised potassium phosphate (0.05 mol/L; pH 6.8) and methanol (58:42, v/v). The flow rate was 0.8 mL/min. Commercial MDA (Sigma–Aldrich; T-8998) was used as an external standard. MDA standards (0.1, 0.5, 1, 2, and 4 μmol/L) were prepared with 1,1,3,3-tetramethoxypropane. The limit of detection was 0.05 μmol/L, the correlation for the linearity of the standard curve was 0.99, and the coefficient of variation was <10% (Lee and Kang, 2008).

Statistical analysis

All statistical procedures were conducted using SAS (ver. 9.1. SAS Institute, Cary, NC). The variables for all biomarkers (1-OHPG, 2-naphthol, and MDA) were logarithm transformed in order to produce normal distributions. To address regional and seasonal variation, all biomarker levels are represented using median and inter-quartile range as well as arithmetic mean and standard error. Correlation analysis was conducted to estimate Pearson correlation