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Urinary polycyclic aromatic hydrocarbon metabolites as biomarkers of exposure to traffic-emitted pollutants

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ABSTRACT

1-*Nitro*-pyrene has been considered a compound specific to diesel combustion emission, while 1- and 2-*nitro*-napthalene are mainly produced through photochemical conversion of naphthalene released to the atmosphere. Metabolites of these compounds may serve as biomarkers of exposure to traffic related pollutants. We collected urine samples from 111 healthy and non-smoking subjects within (i.e., during the Beijing Olympics) and outside (i.e., before and after the Olympics) a traffic control regime to improve Beijing's air quality. Urines were analyzed for the sum of 1&2-*amino*-naphthalene (metabolites of 1- and 2-*nitro*-naphthalene) and 1-*amino*-pyrene (a metabolite of 1-*nitro*-pyrene), using an HPLC-fluorescence method. Within the same time periods, $PM_{2.5}$ mass and constituents were measured, including elemental carbon, sulfate, nitrate, PAHs, carbon monoxide, nitrogen dioxide, sulfur dioxide, ozone, and particle number concentrations. The associations between the urinary metabolites and air pollutants were analyzed using linear mixed-effects models. From the pre- to during-Olympic period, 1&2-*amino*-naphthalene and 1-*hydroxy*-pyrene decreased by 23% ($p = 0.066$) and 16% ($p = 0.049$), respectively, while there was no change in 1-*amino*-pyrene (2% increase, $p = 0.892$). From during- to post-Olympic period, 1&2-*amino*-naphthalene, 1-*amino*-pyrene and 1-*hydroxy*-pyrene concentrations increased by 26% ($p = 0.441$), 37% ($p = 0.355$), and 3% ($p = 0.868$), respectively. Furthermore, 1&2-*amino*-naphthalene and 1-*hydroxy*-pyrene were associated with traffic related pollutants in a similar lag pattern. 1-*amino*-pyrene was associated more strongly with diesel combustion products (e.g. PN and elemental carbon) and not affected by season. Time-lag analyses indicate strongest/largest associations occurred 24–72 h following exposure. 1&2-*amino*-naphthalene and 1-*hydroxy*-pyrene can be used as a biomarker of exposure to general vehicle-emitted pollutants. More data are needed to confirm 1-*amino*-pyrene as a biomarker of exposure to diesel combustion emissions. Controlling creatinine as an independent variable in the models will provide a moderate adjusting effect on the biomarker analysis.

1. Introduction

Nitrated polycyclic aromatic hydrocarbons (*nitro*-PAHs) are known for their mutagenic and carcinogenic toxicities (Talaska et al., 1996). The major sources of atmospheric *nitro*-PAHs include direct emissions from the incomplete combustion of fossil fuels, especially diesel (Feilberg et al., 2001; Bamford and Baker, 2003), and the formation through photochemical reactions between parent PAHs and the hydroxyl radical during daytime and the nitrate radical during nighttime (Atkinson and Arey, 1994; Arey and Arkinson, 2003). Particle-bound PAHs can also be formed through the heterogeneous reactions between PAHs and N_2O_5/NO_3/NO_2 on the surface of particles (Zimmermann et al., 2013). A wide variety of *nitro*-PAHs have been detected in diesel exhaust particles and airborne particles from biomass burning in urban environments (Dimashki et al., 2000; Marino et al., 2000; Bamford and Baker, 2003; Wang et al., 2011). Therefore, people can be exposed to *nitro*-PAHs through the inhalation of airborne particles from diesel and biomass combustion.

Post inhalation, *nitro*-PAHs can be metabolized to *amino*-PAHs that are ultimately excreted through urine (Poirier and Weisburger, 1974; Nachtman and Wei, 1982; van Bekkum et al., 1998). Therefore, it is biologically plausible to use urinary *amino*-PAHs as biomarkers of

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nitro-PAH exposure. A study by Laumbach et al. (2009) observed higher urinary 1-*amino*-pyrene concentrations among human volunteers following a one-hour exposure to a diesel exhaust mixture at 300 μg/m^3 PM_{10} (particulate matter with an aerodynamic diameter smaller than 10 μm) in an exposure chamber, compared to those exposed to clean air (Laumbach et al., 2009). This study supports earlier publications that demonstrated 1-*nitro*-pyrene as one of the *nitro*-PAH isomers to diesel combustion (Schuetzle et al., 1982; Paputapeck et al., 1983; Zwirner-Baier and Neumann, 1999; Bamford and Baker, 2003). In a more recent study, Neophytou et al. (2014) found associations between urinary *amino*-PAHs with vehicle exhaust-related $PM_{2.5}$ (particulate matter with an aerodynamic diameter smaller than 2.5 μm) (Neophytou et al., 2014). Existing studies have also reported increases in urinary 1-*amino*-pyrene in underground miners following a work shift using diesel-powered machinery (Seidel et al., 2002). However, few studies have examined whether urinary *amino*-PAHs are associated with diesel traffic exposure in urban residents.

During the 2008 Beijing Olympics, aggressive air pollution control measures were implemented to reduce traffic emission and improve Beijing's air quality (Wang et al., 2009a). The air pollution control measures included the introduction of new vehicular emission standards, restrictions on diesel-powered vehicles in Beijing's urban areas, limited operation of local industrial and commercial combustion facilities and enforcement of alternate day driving that removed approximately half of the vehicles (~1.5 million) from the local roads each day (Wang et al., 2009a; Rich et al., 2012). Various studies found substantial reductions in traffic-emitted air pollutants, including *nitro*-PAHs, during the Olympic period compared to before and after the Olympics (Wang and Xie, 2009; Wang et al., 2009a, 2011; Rich et al., 2012). Taking advantage of this unique opportunity, we conducted a study to measure urinary 1&2-*amino*-naphthalene and 1-*amino*-pyrene in a panel of Beijing residents (Zhang et al., 2013). By assessing the association between the urinary *amino*-PAHs and exposure to traffic-emitted air pollutants, we aim to evaluate the validity of using 1&2-*amino*-naphthalene and 1-*amino*-pyrene as internal markers of exposure to *nitro*-PAHs in traffic-emitted pollutants. Because 1-*hydroxy*-pyrene as a major metabolite of pyrene has been often used as a biomarker of PAH exposure, we also aim to examine the association between urinary 1-*hydroxy*-pyrene and traffic-emitted pollutants (Strickland and Kang, 1999; Hu et al., 2006).

2. Methods

2.1. Study design and subjects

Centered around the air pollution control measures described above, three sampling periods were defined in our study as: the pre-Olympic period (2 June 2008–19 July 2008) where some relatively mild controls were implemented, the during-Olympic period (July 20, 2008–September 19, 2008) where the full-scale control measures were implemented, and the post-Olympic period (September 20, 2008–October 30, 2008) where the majority of the control measures were relaxed. In previous studies, drastic reductions in the concentrations of air pollutants were observed during the Olympic period compared to the pre- and post-Olympic periods (Wang and Xie, 2009; Wang et al., 2009a, 2010; Rich et al., 2012). Therefore, our three-period study design (the pre-during-post Olympics) followed a 'high-low-high' air pollution changing pattern.

In the current analysis, study subjects included 111 (55 male and 56 female) nonsmoking individuals, 22–27 years of age. The subjects were recruited from the pool of medical residents at the Peking University First Hospital (hereinafter referred to as 'the hospital'). All study participants worked on the campus of the hospital and resided in dormitories at either the hospital or Peking University Health Sciences Center located within 5 km from the hospital. In each of the three Olympic periods, a spot urine sample was collected from each subject in the morning of the visit (between 8 and 10 am). Each subject was scheduled to visit the clinic the same day of the week for all the three visits unless adverse events such as severe respiratory infectious diseases kept the subjects from timely clinical visits (Zhang et al., 2013). The sample collection in each of the three Olympic period lasted for four weeks. The study protocol was approved by the Institutional Review Board of the University of Medicine and Dentistry of New Jersey as well as the Ethics Committee of the Peking University Health Sciences Center and Peking University First Hospital.

2.2. Air pollution monitoring

The air pollution measurements were conducted as described previously (Rich et al., 2012; Zhang et al., 2013). In brief, air samplers and monitors were placed on top of a seven-story building located in the center of the Peking University First Hospital campus. Carbon monoxide (CO), nitrogen dioxide (NO_2), ozone (O_3), $PM_{2.5}$, elemental carbon (EC), sulfur dioxide (SO_2), sulfate (SO_4^{2-}), nitrate (NO_3^-), and particle number concentrations (PN) in the size range of 13.0–764.8 nm were monitored throughout the three study periods, beginning one week before the start of urine sample collection. Ambient temperature and relative humidity (RH) were monitored continuously and concurrently at the same site. $PM_{2.5}$ were collected on a 37 mm Teflon filter using a Quad Channel Ambient Particulate Sampler equipped with an impactor that has an aerodynamic cut-off of 2.5 μm (TH-16A, Tianhong Inc. China) at a flow rate of 16.7 l/min. Polycyclic aromatic hydrocarbon concentrations in $PM_{2.5}$ collected during the pre- and the during-Olympic periods were analyzed from fine particles using a method employing a gas chromatography–mass spectroscopy system (He et al., 2006; Huang et al., 2006). The method details can be found in the Supplementary materials (Appendix I).

2.3. Urinary amino-PAH measurements

The current study used a modified method to analyze urinary *amino*-PAHs as described in a previous publication (Laumbach et al., 2009). In brief, 2 ml of urine sample were incubated with 20 μl of β-glucuronidase from Helix pomatia Type H-2 (Sigma-Aldrich, St. Louis, MO) in 2 ml 0.1 M sodium acetate buffer (pH 5.0) at 37 °C overnight. The hydrolyzed urine samples were adjusted to pH > 10 with the addition of 25 μl of 10 M sodium hydroxide and extracted with 4 ml of ethyl acetate. After mixing on a shaker for 10 min, the samples were centrifuged at 3500 rpm for 10 min. The supernatant was evaporated to dryness under nitrogen in a TurboVap LV evaporator operated at 35 °C. The residue was reconstituted in 200 μl of methanol and 20 μl was injected into an HPLC-fluorescence system for the detection of 1&2-*amino*-naphthalene (as one single peak without resolving 1-AN and 2-AN), 1-*amino*-pyrene, and 1-*hydroxy*-pyrene. The chromatographic separation was achieved on a Supelco-Ascentis RP-Amide column (250 × 4.6 mm, 5 μm, Sigma-Aldrich, St. Louis, MO). The mobile phase was 50% acetonitrile (A) and 100% acetonitrile (B), with a linear gradient from 0% B at 0 min to 70% B at 30 min. The fluorescence detector was set up at 254/425 nm (Ex/Em).

The limits of detection were estimated as 3 times the standard deviation of 8 injections of a lower concentration calibration standard (0.25 ng/ml). The recovery of the assay was expressed as the ratio (%) of the concentration measured to the concentration spiked into a real urine sample (two concentrations were spiked including 1 ng and 10 ng). The precision of the assay was expressed as the coefficient of variation (%) of 8 repeated injections. In summary, the limit of detection of the three PAH metabolites were 0.04, 0.02, and 0.04 ng/ml, the recoveries were 84.3%, 88.3%, and 74.1%, and the precision were 6.0%, 10.1%, and 6.0% for 1&2-*amino*-naphthalene, 1-*amino*-pyrene, and 1-*hydroxy*-pyrene, respectively. The representative chromatograms of unspiked and spiked urine samples were provided in the Supplementary materials (Fig. S8).