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Reproductive hormones in relation to polycyclic aromatic hydrocarbon (PAH) metabolites among non-occupational exposure of males

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ABSTRACT

A limited number of studies have suggested that exposure to PAHs may affect reproductive hormones. Subjects ($n=642$) in this study were from the affiliated hospitals of Nanjing Medical University. Individual exposures to PAHs were measured as spot urinary concentrations of four PAH metabolites, including 1-naphthol (1-N), 2-naphthol (2-N), 2-hydroxyfluorene (2-OF) and 1-hydroxypyrene (1-OP), which were adjusted by urinary creatinine (CR). Blood samples were collected to measure serum levels of reproductive hormones, including follicle-stimulating hormone (FSH), luteotrophic hormone (LH), estradiol (E2), testosterone (T) and prolactin (PRL). All of the subjects had detectable levels of the four metabolites of PAHs in their urine samples. The median concentrations of 1-N, 2-N, 2-OF and 1-OP were 2.440, 4.176, 2.843 and 1.148 μg/g of CR. There were significant *P*-values between increased CR-adjusted 1-N tertiles and E2 concentration, 2-OF tertiles and LH, FSH level, 1-OP and E2 level. The multivariate linear regression results also showed significant correlation between the levels of serum LH and 1-OP (the adjusting *P*-value was 0.048), but no correlations were found between other hormones and the level of PAH metabolites. These observed correlations between levels of hydroxy-PAH and some altered hormones indicated slight endocrine effects on adult men with PAH exposure.

1. Introduction

Endocrine disruption has emerged as an environmental issue based on the hypothesis that exposure to certain environmental chemicals has an effect on hormonal activity and thus alters the endocrine system, increases the incidence of endocrine diseases and disorders, and adversely affects development in both humans and wildlife (Ankley et al., 1997; Colborn et al., 1993; Colborn, 1995; Kavlock, 1999).

Polycyclic aromatic hydrocarbons (PAHs) represent hundreds of compounds with similar chemical structures. PAHs result from incomplete combustion processes and are ubiquitous environmental contaminants and known carcinogens. Some reports show that high detection rates of PAH metabolites among different races and both genders, reflect widespread exposure to parent compounds among the general population (CDC, 2005; Huang et al., 2004; Kamangar et al., 2005; Zhang et al., 2007). Exposure to PAHs among the general population is thought to occur mainly by way of ingestion, inhalation, and dermal contact (Ramesh et al., 2004).

PAHs are extensively metabolized by cytochrome P450 enzymes in humans and animals (Ramesh et al., 2004). The major metabolites of PAHs are monohydroxy phenols (OH-PAHs) and dihydrodiols (Bauer et al., 1995; Burczynski et al., 1998; Conney, 1982; Shimada et al., 1989; Shou et al., 1994; Yun et al., 1992). For the half-life of different PAHs, 1-hydroxypyrene (1-OP) is about 29 h (Huang et al., 2007) in humans. As a consequence of this rapid metabolism, concentrations of PAHs in serum are considerably lower than metabolites in urine. Therefore, biomonitoring of PAH metabolites is an important approach for evaluation of human exposure and body burden of PAHs, particularly 1-OP (Bouchard et al., 1998, 2002; Huang et al., 2007; Mumford et al., 1995; Wu et al., 1998) and hydroxynaphthalenes (Rappaport et al., 2004). These metabolites show estrogenic activities, particularly 1-OP, with potencies much greater than those of the parent compound (Van de Wiele et al., 2005). Our group found that thyroid hormone activity is also related to these metabolites (Sun et al., 2008). However, most studies believe that the AhR activity is the main cause of effects of PAHs and their metabolites (Izawa et al., 2007a; Nebert et al., 2004). Due to limited detection rates of some PAH metabolites and lack of accurate information about PAH exposure in China, we selected four metabolites as our target. They were 1-hydroxynapthalene (1-N, CAS No. 90-15-3, metabolite of naphthalene), 2-hydroxynapthalene (2-N,

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CAS No. 135-19-3, metabolite of naphthalene), 1-OP (CAS No. 5315-79-7, metabolite of pyrene), 2-hydroxyfluorene (2-OF, CAS No. 2443-58-5, metabolite of fluorene).

Many PAHs have been implicated as causative agents of prostate (Kizu et al., 2003), breast (Gammon et al., 2004), pancreatic (Z'graggen et al., 2001), and cervical (Wu et al., 2004) cancers in humans and animal models. Some PAHs or their metabolites are found to bind to the estrogen/androgen receptor and either induce or inhibit the estrogen/antiandrogen response (Santodonato, 1997; Vinggaard et al., 2008; Hirose et al., 2001). The endocrine and reproductive toxicities of PAHs have also been extensively investigated recently (Lee et al., 2007; Seruto et al., 2005; Jeng and Yu, 2008; Izawa et al., 2007b). Animal and limited human studies suggest possible association of PAH exposure with reproductive hormones (Evanson and Van Der Kraak, 2001; Jeng and Yu, 2008; Monteiro et al., 2000; Rocha Monteiro et al., 2000; Kim et al., 2005). However, results of human studies on PAH exposure and male reproductive hormones have been inconsistent, perhaps attributable to differences in geographical locations where research was undertaken, racial differences of subjects, selected biomarkers and exposure levels. Lack of adequate internal monitoring data of different PAHs from multiple routes has also limited understanding of their impact on male endocrine function. To determine whether levels of PAHs in non-occupational exposure males are associated with altered reproductive hormones in adult men, we selected a study population with no specific exposure to compounds with reported male endocrine toxicities. Detection of an association of even small magnitude may have large public health significance because of the widespread distribution of PAHs.

2. Materials and methods

2.1. Subject recruitment

All 642 male participants were diagnosed with unexplained male factor infertility by affiliated hospitals of Nanjing Medical University between Match 2004 and October 2007 (NJMU Infertile Study). The protocol and consent form were approved by the Institutional Review Board of Nanjing Medical University prior to this study. All studies involving human subjects were done under full compliance with governmental policies and the Helsinki Declaration. After explanation of the study procedures and clarification of questions raised, subjects signed informed consent forms. Consecutive eligible men (with wives not diagnosed as infertile) were recruited to participate. Of those approached, 92.8% consented. There were no significant differences in sampling numbers among years and seasons. A detailed physical examination, including height and weight, was performed, and a questionnaire was conducted to collect other information including personal background, lifestyle factors, occupational and environmental exposures, genetic risk factors, sexual and procreate state, medical history and physical activity. Men taking hormonal medications (e.g., Propecia, Finasteride, Cabergoline, Clomid, Gonadotropin-releasing hormone, T, or Prednisone taper) were *a priori* excluded from the study. Those with other known causes related to male endocrine function, for instance, genetic disease and occupational exposure to PAHs, were also precluded. All the participants claimed that their lifestyles and environments had not changed in several months before sample collection. A single spot urine sample was collected from each subject on the same day as the blood sample.

2.2. Measurement of urinary PAH metabolites

Urine specimens were frozen prior to analysis for PAH metabolites. The analyses were basically performed as described by Xu et al. (2004). Briefly, urinary concentrations of PAH metabolites were analyzed by a sensitive and selective LC–MS/MS (Waters 2695 and Waters Premier, USA). Due to low rates of detection, we selected only four metabolites (1-N, 2-N, 1-OP (≥99.0%, Acros®, Gorcanics, New Jersey, England) and 2-OF (≥98.0%, Sigma-Aldich®, USA)) for analysis. The analytes were hydrolysed by using β-glucuronidase/arylsulfatase (98%, Sigma-Aldich®, England) and separated from the matrix by means of a solid-phase extraction [SPE C18 cartridge (Dikma, China)]. Chromatographic separation of monohydroxylated PAHs was performed on a XterraC18 (2.1 mm*150 mm,5 μm, Waters, USA) using a gradient from 60% aqueous phase (45% methanol) to 100% methanol 23 min after being held at 10% aqueous phase for 0.1 min. The column was then re-equilibrated using 60% aqueous for 4 min. The flow rate of the mobile phase was 0.15 mL/min. Mass spectrometric analyses were performed using a Quantum triple quadrupole mass spectrometer (Waters, USA) equipped with an electrospray ionization (ESI) interface, operated in a negative ion mode with a spray voltage of −2.6 kV. The capillary temperature was set at 125 °C. The mass spectrometer was operated in the multiple reaction monitoring (MRM) modes. 1-N and 2-N monitored the parent ion of 143 (m/z) and 115 (m/z); 2-OF monitored the parent ion of 181 (m/z) and 153 (m/z); 1-OP monitored the parent ion of 217 (m/z) and 189 (m/z). The calibration was carried out using pooled urine to which known amounts of PAH metabolites were added, processed and analyzed in the same manner as the samples. The correlation appeared to be linear between 1 and 100 μg/L of PAH metabolites ($r>0.99$). The limit of detection for 1-OP was 0.15 μg/L, for 1-N, 2-N and 2-OF was 0.3 μg/L. The relative standard deviation (RSD) of the within-series imprecision was between 3.3% and 12.3% at a spiked concentration of 3, 8 and 80 μg/L and the relative recovery was between 80.8% and 122.7% ($n=5$), depending on different spiked concentrations. The levels of PAH metabolites were adjusted using creatinine (CR) concentrations to correct for variable urine dilution in the "spot" urine samples. Samples with CR concentrations above 300 mg/dL or below 30 mg/dL were considered too concentrated or too diluted to provide valid results and were excluded from the primary analysis (Teass et al., 1998). CR concentrations in urine were determined using a commercially available diagnostic enzyme method (WAKO, Japan; 7020 Hitachi, Japan). Quality control samples were analyzed in parallel with unknown samples.

2.3. Stabilities of urinary PAH metabolites

According to the half-life of PAHs, we collected consecutive urina sanguinis from six healthy young men on the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 9th, 11th, and 13th days over two weeks to evaluate variabilities of the four PAH metabolites. During these days, the donors did not change their lifestyles or environments.

The urinary samples were divided into two groups (including seven sampling days, respectively): Group A for every other day stability analysis, Group B for every day stability analysis. CR concentrations were used to adjust for variable urine dilution in all samples when measuring PAH metabolites. The coefficient of variability (CV) was used to suggest stabilities of the metabolites in human urine.

2.4. Reproductive hormones analysis

Blood samples were drawn between the hours of 9 a.m. and 4 p.m. to avoid the highest hormone secretion time span from 6 a.m. to 8 a.m. on the same day when the urine samples were collected. The serums were put in a centrifuge and frozen at −70 °C until used. The serum levels of follicle-stimulating hormone (FSH), luteotrophic hormone (LH), estradiol (E_2), testosterone (T) and prolactin (PRL) were measured by radioimmunoassay (RIA) by using commercial RIA kits (Beijing North Institute of Biological Technology, China). Four internal and external qualitatively controlled samples were included in each assay run. Detection limits for FSH and LH were 1.0 IU/L and 0.5 IU/L, and those for E2, T and PRL were 4 pg/mL, 0.35 ng/mL and 6.5 μIU/mL, respectively. The mean intra- and inter-assay coefficients of variation