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Polycyclic aromatic hydrocarbons exposure decreased sperm mitochondrial DNA copy number: A cross-sectional study (MARHCS) in Chongqing, China☆

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental pollutants that have adverse effects on the male reproductive function. Many studies have confirmed that PAHs preferentially accumulate in mitochondria DNA relative to nuclear DNA and disrupt mitochondrial functions. However, it is rare whether exposure to PAHs is associated with mitochondrial damage and dysfunction in sperm. To evaluate the effects of PAHs on sperm mitochondria, we measured mitochondrial membrane potential (MMP), mitochondrial DNA copy number (mtDNAcn) and mtDNA integrity in 666 individuals from the Male Reproductive Health in Chongqing College Students (MARHCS) study. PAHs exposure was estimated by measuring eight urinary PAH metabolites (1-OHNap, 2-OHNap, 1-OHPhe, 2-OHPhe, 3-OHPhe, 4-OHPhe, 2-OHFlu and 1-OHPyr). The subjects were divided into low, median and high exposure groups using the tertile levels of urinary PAH metabolites. In univariate analyses, the results showed that increased levels of 2-OHPhe, 3-OHPhe, ∑Phe metabolites and 2-OHFlu were found to be associated with decreased sperm mtDNAcn. After adjusting for potential confounders, significantly negative associations of these metabolites remained ($p = 0.039$, 0.012, 0.01, 0.035, respectively). Each 1 μg/g creatinine increase in 2-OHPhe, 3-OHPhe, ∑Phe metabolites and 2-OHFlu was associated with a decrease in sperm mtDNAcn of 9.427%, 11.488%, 9.635% and 11.692%, respectively. There were no significant associations between urinary PAH metabolites and sperm MMP or mtDNA integrity. The results indicated that the low exposure levels of PAHs can cause abnormities in sperm mitochondria.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are generated from the incomplete combustion of organic material, automobile exhaust, cigarette smoking, coal burning, home cooking, and industrial production processes, and they inevitably enter the human body through inhalation, ingestion, and dermal contact. Because of the widespread availability of PAHs in the environment, the great potential for human exposure, and the mutagenicity and carcinogenicity detected in animal experiments or human epidemiological investigations, many chemicals of PAHs are classified as probable human carcinogens by the International Agency for Research on Cancer (IARC). Moreover, exposure to PAHs or their reactive intermediates has been found to affect the integrity of reproductive functions in mammals, and it ultimately contributes to male infertility. In particular, both epidemiological studies and animal experiments have reported that increased PAHs levels were associated with poor sperm quality, and a correlation between exposure to PAHs and an increased risk of male idiopathic infertility was also observed (Chen et al., 2011; Han et al., 2011; Jeng et al., 2013; Raychoudhury and Kubinski, 2003; Xia et al., 2009b; Xu et al., 2014). However, the potential impact of exposure to PAHs on human fertility remains controversial and inconclusive, this uncertainty may be attributed to between-study differences in the

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studied regions, races, selected biomarkers, exposure levels and multiple routes of PAHs (Xia et al., 2009a). Especially among the general populations exposed at low levels, the weak correlation between PAHs exposure and reproductive damage might not be identified by conventional semen quality detection.

To assess the reproduction risk from exposure to PAHs, it is essential to quantify the actual dose of chemicals that absorbed by the body (Tuntawiroon et al., 2007). PAHs are rapidly metabolized in biological systems, and the concentrations of PAHs in serum are lower than their metabolites excreted in urine (Jeng et al., 2013). After PAHs enter the body, they are predominantly transformed into soluble intermediates through cytochrome P450 enzymes, subsequently formed conjugates with sulfate, glutathione (GSH) or glucuronic acid, and then the conjugated metabolites are primarily eliminated from the body in the urine. Thus, biomonitoring of urinary PAH metabolites is an important approach to estimate the human exposure to and the body burden of PAHs. Most intermediates of PAHs are electrophilic and capable of covalently binding to DNA to form PAH-DNA adducts. PAH−DNA adducts were found to inhibit meiotic division during spermatogenesis and could be associated with infertility (Gaspari et al., 2003). In addition to forming bulky DNA adducts in the nucleus, PAHs have been shown to have greater affinity for mitochondrial DNA (mtDNA) than nuclear DNA (Allen and Coombs, 1980; Backer and Weinstein, 1982), and they have the potential to inhibit mitochondrial DNA synthesis (Stairs et al., 1983). Studies have further reported that PAHs exposure is associated with abnormal mtDNA copy number (mtDNAcn) in the blood of both occupational and general populations (Pavanello et al., 2013; Pieters et al., 2013), indicating that mtDNA may be one of the genetic targets injured by PAHs. However, until now no data have described the potential effect of PAHs exposure on sperm mtDNA.

Mitochondria, which produce adenosine triphosphate (ATP) through oxidative phosphorylation (OXPHOS), play an important role in critical cellular functions including energy metabolism, calcium homeostasis, reactive oxygen species (ROS) generation and apoptosis. During spermatogenesis and fertilization, sperm biosynthesis and motility require ATP, suggesting that sperm mitochondrial function may be important for flagellar propulsion and sperm fertilization capacity (Cummins, 2004; Rajender et al., 2010). Because the bioenergetic function of mitochondria is crucial for semen quality, particularly sperm motility, any quantitative or qualitative aberrations in mitochondria may affect the cellular functioning of spermatozoa (Kumar and Sangeetha, 2009). More recently, sperm mtDNAcn and mtDNA integrity, two major mitochondrial genetic features, have also been studied in the context of male infertility. Song and Lewis (2008) have observed a significant increase in mtDNAcn and a decrease in mtDNA integrity in men with poor semen quality. May-Panloup et al. (2003) have reported significant increases in mtDNAcn in spermatozoa from men with abnormal semen parameters. Moreover, a decrease in sperm mtDNA content was detected in patients with asthenospermia or poor sperm motility (Kao et al., 2004). These data indicate that mtDNA may be a sensitive predictor of male infertility. Unlike nuclear DNA, mtDNA is particularly susceptible to various kinds of damage due to the surrounding of high levels of cellular ROS, lack of protective histones and limited DNA repair capacity. Internal or external factors, such as ROS and DNA adducts, may directly injure mtDNA and cause a high rate of mutations (Masayesva et al., 2006; Stepanov and Hecht, 2009). As a result, cells with defective mitochondria will synthesize more mtDNA copies to compensate for the damage. Alternatively, defective mitochondria can be eliminated through the mechanisms of autophagy or fission/fusion of mitochondria, leading to the decrease of mtDNA content (Zhang et al., 2007). Therefore, mitochondrial damage and dysfunction in sperm may represent an adverse effect of PAHs on the male reproduction.

In the present study, we investigated the relationship between urinary PAHs metabolites and sperm mitochondria in male participants from the Male Reproductive Health in Chongqing College Students (MARHCS) prospective cohort study. PAHs exposure was characterized by measuring the corresponding urinary metabolites, and its effects on mitochondria were quantified through analyses of MMP, mtDNAcn and mtDNA integrity in the sperm of each subject.

2. Materials and methods

2.1. Study population

The current study is part of a perspective cohort “MARHCS” (Male Reproduction Health in Chongqing College Students). The MARHCS study was designed to investigate the influence of environmental and socio-psycho-behavioral factors on male reproductive health. The baseline measurements were performed at the university town of Chongqing in June 2013. After September 2013, some of the students moved to the urban campus, and the remaining students stayed in the university town due to the lecture schedules of the college. The participants were followed up for the first time in June 2014. The following inclusion criteria were applied: sophomores (older than 18 years of age) studying in the university town of Chongqing city, with an abstinence period between 2 and 7 days. Participants who had a history of reproductive or urologic diseases were excluded. All eligible participants were asked to sign informed consent forms, complete a unified questionnaire, undergo physical examinations and provide biological samples (semen, blood, and urine) collection. The current study was established at the first follow-up stage in 2014. The study protocol and consent form were approved by the Ethical Committee of the Third Military Medical University. More detailed information about the study can be found elsewhere (Yang et al., 2015).

2.2. Urinary PAH metabolites measurement

Urine samples were collected with glass devices to avoid contamination and then stored at −20 °C until analysis. The concentrations of the eight PAH metabolites [1-hydroxynaphthalene (1-OHNap), 2-hydroxynaphthalene (2-OHNap), 1-hydroxyphenanthrene (1-OHPhe), 2-hydroxyphenanthrene (2-OHPhe), 3-hydroxyphenanthrene (3-OHPhe), 4-hydroxyphenanthrene (4-OHPhe), 2-hydroxyfluorene (2-OHFlu) and 1-hydroxypyrene (1-OHPyr)] in urine (available samples n = 492) were analyzed using high-performance liquid chromatography with electrospray triple-quadrupole tandem mass spectrometry (HPLC-MS/MS). Briefly, the urine samples were aspirated into β-glucuronidase and arylsulfatase (Sigma-Aldrich, Inc., St Louis, MO, USA), and purified using C18 solid phase extraction (SPE) cartridges. The extracts were concentrated under a stream of nitrogen gas to dry and then separated from the other urine components using Agilent 1260 high-performance liquid chromatography, and finally, detected using an Agilent G6420 triple quad mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The average recovery for the eight metabolites ranged from 85.6% to 118.3%. The limit of detection (LOD) for the metabolites ranged from 0.012 to 0.058 ng/ml. The relative standard deviation (RSD) of the within-series imprecision was between 2.9 and 10.1%. Urinary creatinine (CR) concentrations were measured in all samples using an automated chemistry analyzer (Shimazu CL-8000; Shimazu, Inc., Tokyo, Japan) to adjust the PAH concentrations.