



Transfer of polycyclic aromatic hydrocarbons from mother to fetus in relation to pregnancy complications

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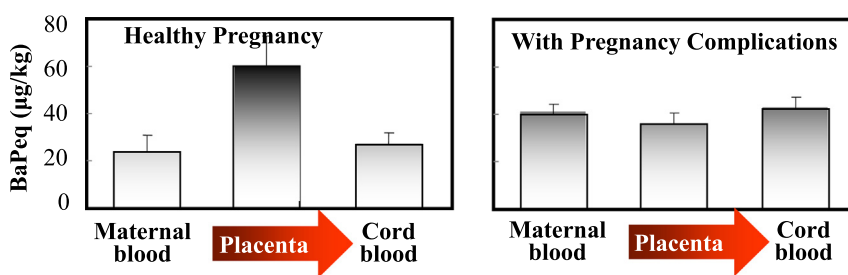
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HIGHLIGHTS

- High-molecular-weight PAH exposure predominate in pregnant women in Kunming.
- $\Sigma_{\text{HMW}}\text{PAHs}$ in control group is higher in maternal blood than in umbilical cord blood.
- $\Sigma_{\text{HMW}}\text{PAHs}$ in the placenta is higher in the control group than in the case group.
- The same results are observed after the exclusion of the impact of the genotypes.
- PAHs accumulating inside the placenta decreases PAH transfer from mother to fetus.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 6 February 2018

Received in revised form 20 April 2018

Accepted 20 April 2018

Available online xxxx

Editor: Jay Gan

Keywords:

Polycyclic aromatic hydrocarbons

Mother–fetus transfer

Pregnancy complications

Placenta function/barrier

Genetic polymorphism

ABSTRACT

The accumulation and transfer of carcinogens, including polycyclic aromatic hydrocarbons (PAHs), in the human body, especially from mother to fetus, has been the subject of many research studies, but the related data are limited and the mechanisms are unknown. This is the first study to investigate the distribution of PAHs in paired samples of maternal blood, placenta tissue, and umbilical cord blood in relation to pregnancy complications. Sixty-four pairs of samples were collected in Kunming, China; 18 were from healthy pregnant women and 46 were from patients with pregnancy complications. The predominant PAHs in these pregnant women were high-molecular-weight (HMW) compounds, mainly from the incomplete combustion or pyrolysis of biomass. In the control group, the total amount of HMW compounds ($\Sigma_{\text{HMW}}\text{PAHs}$) was significantly higher in maternal blood than in umbilical cord blood, which suggested that placenta may decrease PAH transfer in healthy pregnant women. However, this phenomenon was not observed for low-molecular-weight PAHs or in the case group. In the control group, $\Sigma_{16}\text{PAH}$ and $\Sigma_{\text{HMW}}\text{PAH}$ in the placenta were higher than those in maternal blood and umbilical cord blood; for the case group, a contrasting result was observed. $\Sigma_{\text{HMW}}\text{PAHs}$ in the placenta was significantly higher in the control group than in the case group. The same results were observed after the exclusion of the impact of the genotypes of the PAH metabolic enzymes (both phase I and phase II enzymes). Thus, the decreased PAH transfer from mother to fetus may partially result from the accumulation of PAHs inside the placenta.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are formed during the incomplete combustion of organic materials, such as coal or fossil fuel

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combustion, biomass burning, car exhaust, forest fires, waste incineration, and smoke exhaust (Abdel-Shafy and Mansour, 2016; Kim et al., 2013). They are widespread in the environment and smoked or fired foods (Kamankesh et al., 2015; Olatunji et al., 2014). PAHs are lipophilic compounds that may be rapidly accumulated by organisms. Thus, PAHs have been widely detected in the environment and food, which subsequently resulted in their accumulation in human blood, placenta, and other tissues, organs, or body fluids (Anastasia et al., 2015; Ke et al., 2017; Londoño et al., 2015; Machado et al., 2014; Mastovska et al., 2015). PAHs have been identified as carcinogenic, mutagenic, and teratogenic compounds that exert neurotoxicity, immunotoxicity, and genetic and developmental toxicity (Gao and Burchiel, 2014; Jedrychowski et al., 2005; Perera et al., 2012; Yang et al., 2010).

Because of these significant health risks, PAHs have been listed as priority pollutants by many countries. These chemicals are not easily degraded and thus their accumulation and transfer to the human body are of particular interest in research. The maternal-fetal system is very sensitive to environmental stresses, with maternal blood, the placenta, and umbilical cord blood acting as important indicators of maternal/fetal PAH exposure and the transfer from mother to fetus (Crowell et al., 2013; Zhang et al., 2017). The pathological pregnancies, such as hypertensive disorder, diabetes during pregnancy, and preterm delivery, may disturb the normal physiological processes and consequently alter PAH transfer in the human body. However, owing to the limited availability of samples, PAHs in maternal blood, the placenta, and umbilical cord blood have been investigated in only several studies to assess the health risks of maternal or fetal exposure to PAHs (Chen et al., 2014; Tsang et al., 2011; Yu et al., 2011; Zhang et al., 2017). Even in these limited studies, the investigators generally focused on maternal blood (Wang et al., 2015), the placenta (Gladden et al., 2000; Singh et al., 2008b), or umbilical cord blood (Guo et al., 2012), and seldom with paired samples of maternal blood, the placenta, and umbilical cord blood. Zhang et al. (2017) presented a report on paired samples (maternal and umbilical cord serum, and placenta), which mostly focused on the influence of lipophilicity on PAH transfer from mother to fetus. It was therefore unable to fully explain the exposure of PAHs in the maternal-fetal system and the effect of pregnancy complications on the placental transfer of PAHs from the mother to the fetus. This study selected regular long-term residents of Kunming as the research subjects, which aimed to investigate (1) the concentrations and distribution patterns of PAHs in pregnant women in Kunming area; and (2) the potential for PAH transfer from the mother to the fetus in relation to pregnancy complications.

2. Materials & methods

2.1. Study population and sample collection

This project was approved by the Medical Ethics Committee of the First People's Hospital of Yunnan Province. Sixty-four pregnant women, who did not smoke, abuse drugs or alcohol, or had a history of occupational exposure, were investigated in Kunming. These women attended the antenatal clinic and delivery room in the Department of Obstetrics of the First People's Hospital of Yunnan Province between 2013 and 2015. Women who agreed to participate signed an informed consent document. We divided the cases into two groups based on maternal pregnancy complications or delivery outcome. The control group ($n = 18$) included women without pregnancy complications and that delivered at full-term. The case group ($n = 46$) included women with hypertensive disorder, diabetes during pregnancy, or preterm delivery (gestational age < 37 weeks). According to the pathological degree, the hypertensive disorder complications were divided into gestational hypertension, mild preeclampsia, and severe preeclampsia.

Approximately 10 mL of maternal venous blood, 10 mL of umbilical cord blood, and 30–50 g of placenta were sampled immediately after delivery by well-trained midwives or obstetricians. All samples were labeled, transported to the laboratory under freezing conditions,

processed, and stored at $-80\text{ }^{\circ}\text{C}$ until the samples were used for the experiments.

The information on maternal age, height, weight, gravidity, parity, medical history, pregnancy complication diagnosis, ethnic group, place of residence, infants' birth date, Apgar scores, and gestational age was obtained from medical records. Other information, including educational level, smoking, alcohol consumption, drug use, occupation, potential environmental exposure, and lifestyle, was obtained using a questionnaire at the time of enrolment.

2.2. Chemicals and reagents

Chromatographic grade *n*-hexane, dichloromethane, methanol (purchased from Aladdin, Shanghai, China) and ultrapure water were used in the PAH extraction and sample cleanup procedures. To remove water, anhydrous sodium sulfate was used in the extraction and purification processes. A composite standard solution of 16 priority PAHs suggested by the United States Environmental Protection Agency (USEPA) including naphthalene (Nap), acenaphthylene (Any), acenaphthene (Ana), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), benzo(a)anthracene (BaA), chrysene (Chr), benzo(a)pyrene (BaP), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), indeno(1,2,3-cd)pyrene (InP), dibenzo(a,h)anthracene (DbA), and benzo(g,h,i)perylene (BghiP) at 2000 mg/L was obtained from ANPEL Laboratory Technologies (Shanghai, China).

2.3. PAH extraction and sample purification

The extraction method and cleanup procedure were based on several previous studies, with some modifications (Kishikawa et al., 2003; Olatunji et al., 2014). Two milliliters of maternal peripheral blood or umbilical cord blood, or 5–10 g placenta was placed in a 40-mL round-bottomed flask. For saponification, each sample was mixed with 6 mL (for blood samples) or 10 mL (for placenta samples) of 0.4 mol/L sodium hydroxide in ethanol-water (9:1, v/v) mixture. The resulting mixture was refluxed in a water bath at $70\text{ }^{\circ}\text{C}$ for 90 min to allow complete saponification. Subsequently, 20 mL of a mixture of dichloromethane and *n*-hexane (1:1, v/v) was added to the saponification products, which were extracted under ultrasonication for 30 min, centrifuged at 1500g for 15 min, and the supernatant was collected. This procedure was repeated three times to ensure full PAH recovery. The extracts were combined and 2 g of anhydrous sodium sulfate were added to remove water. The samples were reduced to a volume of 2 mL by using a rotary evaporator to at $19 \pm 2\text{ }^{\circ}\text{C}$ (room temperature).

The concentrated extracts were passed through solid-phase extraction (SPE) columns to remove co-extracted lipids and impurities that would otherwise interfere with the subsequent PAH qualification and quantification. The purification methods used were similar to those reported in the literature (Guo et al., 2012; Singh et al., 2008a). Briefly, SPE cartridges (RP-18) were washed with 6 mL of a mixture of *n*-hexane and dichloromethane (1:1, v/v) and activated with 3 mL ultrapure water. The samples were then loaded on cartridges and the extraction solution was aspirated through the cartridge, which rested on a portable positive air pump with a flow rate of $\leq 1\text{ mL/min}$. The solid phase was washed with 4 mL ultrapure water, dried, and then eluted with 4 mL of a mixture of *n*-hexane and dichloromethane (1:1, v/v). A sufficient amount of anhydrous sodium sulfate was added to the effluent to remove water, and the effluent was concentrated to 200 μL under a gentle stream of nitrogen. The samples were transferred to amber gas chromatography (GC) vials for analysis by GC-mass spectrometry (MS).

2.4. Instrumental analysis of PAHs

The purified samples were subjected to GC-MS for the 16 priority PAHs. Five microliters of cleaned extracts were injected into a HP-5MS capillary column (Agilent) (30 m \times 0.25 mm i.d. \times 0.25 μm film

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