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Quasi-targeted analysis of hydroxylation-related metabolites of polycyclic aromatic hydrocarbons in human urine by liquid chromatography–mass spectrometry

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ABSTRACT

Metabolite identification is crucial for revealing metabolic pathways and comprehensive potential toxicities of polycyclic aromatic hydrocarbons (PAHs) in human body. In this work, a quasi-targeted analysis strategy was proposed for metabolite identification of monohydroxylated polycyclic aromatic hydrocarbons (OH-PAHs) in human urine using liquid chromatography triple quadruple mass spectrometry (LC–QqQ-MS/MS) combined with liquid chromatography high resolution mass spectrometry (LC–HRMS). Potential metabolites of OH-PAHs were preliminarily screened out by LC–QqQ-MS/MS in association with filtering in a self-constructed information list of possible metabolites, followed by further identification and confirmation with LC–HRMS. The developed method can provide more reliable and systematic results compared with traditional untargeted analysis using LC–HRMS. In addition, data processing for LC–HRMS analysis were greatly simplified. This quasi-targeted analysis method was successfully applied to identifying phase I and phase II metabolites of OH-PAHs in human urine. Five metabolites of hydroxynaphthalene, seven of hydroxyfluorene, four of hydroxyphenanthrene, and three of hydroxypyrene were tentatively identified. Metabolic pathways of PAHs in human body were putatively revealed based on the identified metabolites. The experimental results will be valuable for investigating the metabolic processes of PAHs in human body, and the quasi-targeted analysis strategy can be expanded to the metabolite identification and profiling of other compounds in vivo.

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1. Introduction

Liquid chromatography–mass spectrometry technologies (LC–MS) have been developed rapidly in the last decades [1–3], which has greatly facilitated metabolite identification and profiling for drugs in drug discovery and development industries [4,5]. In addition, these technologies have also become mainstream tools for the emerging metabolomics [6–9]. Recently, they have been applied to identifying and profiling metabolites and transformation products of environmental pollutants [10–12], promoting studies concerning in vivo and in vitro metabolism/transformation of pollutants.

LC coupled with triple quadruple mass spectrometry (LC–QqQ-MS/MS) operated in multiple-reaction monitoring (MRM) mode is commonly used for targeted analysis. Moreover, it can also be used for tentatively untargeted metabolite identification of xenobiotic compounds via precursor ion scan (PIS), product ion scan, neutral loss scan (NLS), and MRM modes [13]. Some metabolites, e.g., phase II metabolites, can generate chromatographic peaks in MRM channels set for monitoring their parent compounds [14,15]. In addition, mass-to-charge (m/z) values of precursor ions of potential metabolites, usually quasi-molecular ions can be obtained in PIS mode. Furthermore, NLS is also a useful approach for confirming metabo-

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lites, particularly for phase II metabolites such as conjugates of glucuronide, sulfate and glutathione [16]. By means of these scan modes and referring to predicted in vivo metabolic logics of xenobiotics, potential metabolites can be preliminarily screened out. However, accuracy and reliability of the identification could not be guaranteed due to the low mass-resolving power of LC–QqQ-MS/MS.

LC equipped with high resolution mass spectrometry (LC–HRMS), e.g., LC quadrupole time-of-flight MS (LC–QTOF-MS) and LC–Q-Orbitrap-MS, operated in full scan mode has been widely used for untargeted metabolite identification due to its accurate mass determination [13,17]. However, data mining processes are complex and time-consuming, let alone potential false positives [18,19]. Fortunately, combinatorial utilization of LC–QqQ-MS/MS and LC–HRMS can be a promising solution for metabolite identification due to the integrated technical advantages and avoided limitations [20–22].

Polycyclic aromatic hydrocarbons (PAHs) are a group of the most widespread pollutants in the environment. Once entering human body, PAHs are possibly subjected to successive phase I metabolic biotransformations including oxidation, hydroxylation, and hydration, which are catalyzed by cytochrome P450 dependent monooxygenases, giving rise to a variety of epoxy derivatives [23,24]. *In vivo* hydrolysis of PAHs occurs via catalysis of hydrolase [25], producing a variety of dihydrodiol epoxides which are considered to be carcinogenic due to their ability to form DNA adducts through covalent bonds [26,27]. Some of the phase I metabolites, such as monohydroxylated PAHs (OH-PAHs) have been frequently detected in human urine and hair [28–30]. Urinary OH-PAHs, particularly 1-hydroxypyrene (1-OHP), have been used as biomarkers to indicate human exposure to PAHs [31–34]. Therefore, investigation of urinary OH-PAHs has attracted increasing attentions in environmental studies [35,36]. OH-PAHs may further undergo phase I metabolisms (e.g., oxidation and hydration) and phase II metabolisms (such as glucuronidation and sulfation), generating various phase I and phase II metabolites, e.g., polyhydroxylated PAHs, PAH dihydrodiols, and glucuronide conjugates and sulfate derivatives of OH-PAHs. Thus, a variety of both phase I and phase II metabolites of OH-PAHs may be concurrent in human body due to complex *i*n vivo metabolic reactions [23]. Identification and profiling of metabolites of OH-PAHs are therefore of critical importance to explore and elucidate metabolic pathways of PAHs in human body. Previous studies have revealed presence of some metabolites of OH-PAHs, such as glucuronides and sulfates in human urine [37–42]. However, detailed identification and profiling of OH-PAHs metabolites in human body have not been reported yet.

In this work, we conducted a study using quasi-targeted analysis for tentative metabolite identification and profiling of OH-PAHs in human urine. Potential metabolites of OH-PAHs were preliminarily screened out by LC–QqQ-MS/MS combined with filtering in an information list of possibly existent in vivo metabolites of OH-PAHs, and further confirmed by targeted identification approach using LC–QTOF-MS and LC–Q-Orbitrap-MS. A variety of metabolites of OH-PAHs were identified and the corresponding metabolic pathways were subsequently elucidated. The results will be valuable in elucidation and interpretation of in vivo metabolism of PAHs in organisms.

2. Experimental

2.1. Chemicals and materials

Authentic standards 2-hydroxynaphthalene (2-OHN, purity: 99%), 3-hydroxyfluorene (3-OHF), 2-hydroxyfluorene (2-OHF, purity: 98%), 9-hydroxyfluorene (9-OHF, purity: 96%), 9-hydroxyphenanthrene (9-OHPhe), and 1-hydroxypyrene (1-OHP, purity: 98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 1-Hydroxyphenanthrene (1-OHPhe, purity: 99%), 2-hydroxyphenanthrene (2-OHPhe, purity: 99.6%), and 4-hydroxyphenanthrene (4-OHPhe, 50 μg/mL in acetonitrile) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). 1-Hydroxynaphthalene (1-OHN, purity: 99%) and 3-hydroxyphenanthrene (3-OHPhe, purity: 98%, 50.0 μg/mL in toluene) were purchased from Fluka (St. Louis, MO, USA) and Cambridge Isotope Lab (Andover, MA, USA), respectively. Structures of the chemicals are illustrated in Fig. S-1 and the physicochemical properties are listed in Table S-1. HPLC-grade methanol (MeOH) and acetonitrile (ACN) were purchased from Merck Crop. (Darmstadt, Germany). Ammonium acetate (NH_4Ac) was of HPLC grade and purchased from J&K Scientific Ltd. (Beijing, China). Ultrapure water (electrical resistivity of 18.2 MΩ cm) was prepared with a Millipore water purification system (Millipore Corporation, Bellerica, MA, USA).

Stock solutions of the OH-PAHs were individually prepared in ACN with concentrations from 1.2 to 3.6 mg/mL. A neat solution containing all the OH-PAH standards was prepared with 0.5 mL of MeOH/H_2O (1:1, v/v) and used as the working solution for peak identification and referencing. All standard solutions were kept in a freezer at −20 °C before use.

2.2. Urine samples

Urine samples were collected from occupational exposure populations in two coke plants in Qujing, Yunnan Province, China and non-occupational exposure farmers living in the vicinity of the coke plants. The famers were expectedly exposed to PAHs stemming from biomass burning and coke plants. One hundred coke plant workers including 72 males and 28 females with ages of 36.0 ± 11.0 years were recruited for urine collection. In addition, 14 male and 11 female famers were recruited with ages of 34.9 ± 9.5 years. Other personal and life style information such as living address, job responsibilities in coke plants, cooking styles, smoking, alcohol drinking, and dietary habits have been provided in detail previously [43].

Recruited persons were required to avoid from consuming baked/deep-fried food for three days prior to urine collection in order to exclude additional PAHs exposure. Samplings were conducted in December 2012. Urine samples were collected in polypropylene bottles sealed with screw caps and immediately frozen with dry ice before transport to the laboratory where the urines were stored at −80 °C prior to treatment.

All the urine samples were firstly subjected to quantitative analysis of OH-PAHs with commercially available standards as described in detail previously [43]. Five urine samples that contained relatively high concentrations of the OH-PAHs were subsequently selected and pooled as a composite sample for metabolite identification and profiling in this study. Average concentrations of the quantified OH-PAHs in these five urine samples were 1.32–19.20 ng/mL as shown in Table S-2.

2.3. Sample treatment

Aliquot 800 μL of the composite urine sample was placed into a 2-mL polypropylene tube followed by addition of 800-μL MeOH. The sample was vortex-mixed for 5 min and then subjected to centrifugation at 15800g and 4 °C for 30 min using a Thermo Multifuge X3R centrifuger (Thermo-Fisher Scientific, Waltham, USA). After centrifugation, 800 μL of the supernatant was transferred to a clean amber glass vial and subjected to instrumental analysis.

The urine sample was not treated with enzymatic hydrolysis using β-glucuronidase and arylsulfatase in order to keep all

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