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Urinary *trans-anti*-7,8,9,10-tetrahydroxy-7,8,9,10-tetrahydrobenzo(*a*) pyrene as the most relevant biomarker for assessing carcinogenic polycyclic aromatic hydrocarbons exposure

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous pollutants present as complex mixtures in the environment. Among them, benzo(*a*)pyrene (BaP) is classified as carcinogenic to humans by the International Agency of Research on Cancer. Taking into account all absorption ways, human biomonitoring allows PAH exposure assessment, but biomarkers both specific to carcinogenic effect and sufficiently sensitive are lacking. In this work, we proposed the urinary 7,8,9,10-tetrahydroxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene (7,8,9,10-OHBaP) stemming from hydrolysis of BaP-7,8-diol-9,10-epoxide, the ultimate carcinogenic BaP metabolite, as biomarker of PAH exposure. A simple and highly sensitive analytical method, with a limit of quantification (LQ) reaching 0.06 pmol/L (0.02 ng/L), was described and validated. The relevance of urinary 7,8,9,10-OHBaP concentrations adjustment by creatinine was demonstrated. In a group of 24 non-occupationally PAH exposed subjects, only 15% of 7,8,9,10-OHBaP levels was below the LQ and the last daily void has been found as the best sampling time. Tobacco consumption had a significant positive effect on 7,8,9,10-OHBaP concentrations with a 90^{e} percentile equal to 0.05 nmole/mole creatinine (nmol/mol) and 0.03 nmol/mol for smokers and non-smokers, respectively. In case of occupational PAH exposure, all the pre- and post-shift urinary 7,8,9,10-OHBaP levels of 7 non-smoking workers in a prebaked electrodes production plant were above the LQ. Concentrations ranged from 0.05 to 0.91 nmol/mol and accumulation of 7,8,9,10-OHBaP into organism of workers during the working week was clearly observed. The best sampling time was the post-shift at the end of week but samples should also be collected at pre-shift the beginning of week to assess the background level. Finally, the urinary 7,8,9,10-OHBaP elimination kinetic through the weekend was studied using non-linear mixed effect modelling. Mean apparent urinary half-life was 31.5 h with low inter-individual variability. Describing key characteristics of urinary 7,8,9,10-OHBaP as PAH exposure biomarker, this work should promote its use for future large-scale biomonitoring campaigns.

1. Introduction

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous pollutants produced by incomplete combustion of organic matter and coal or petroleum distillation. While several particulate PAH are classified as probably or possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC), benzo(*a*)pyrene (BaP) is the only PAH classified as a known human carcinogen (IARC, 2010). Urban pollution, tobacco smoke and grilled, smoked, fried or contaminated food are responsible for population exposure (Menzie et al., 1992). Industrial processes as coal-tar distillation, paving and roofing with coal-tar pitch or aluminum production cause lung, skin and bladder cancers due to high occupational PAH exposure (IARC, 2012). While respiratory uptake is often the main absorption way, dermal exposure may also be an important route for occupational exposure (Van Rooij et al., 1993).

Biomonitoring of exposure taking into account all absorption routes, occupational and leisure exposure, and personal protective equipment

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efficiency, is more accurate than atmospheric PAH measurements. PAH metabolites are largely used as exposure biomarkers (Brandt and Watson, 2003; Jongeneelen, 1997). These metabolites are conjugated to sulfate or glucuronic acid before their excretion in feces and urine (Jacob and Seidel, 2002). Urinary analyses remain the gold standard because metabolites accumulate in the bladder between two voids and urine is very easy to collect (Smolders et al., 2009).

Urinary 1-hydroxypyrene (1-OHP) was the first proposed PAH exposure biomarker (Jongeneelen et al., 1985). It is still being used because its relatively larger amounts in comparison with other metabolites of PAH (around μg/L) facilitate its analysis (Hansen et al., 2008). However, its levels are influenced by both vapor and particulate atmospheric pyrene indifferently (Barbeau et al., 2015). Urinary monohydroxylated metabolites of gaseous PAH are also often used for assessing individual exposure (Barbeau et al., 2017; CDC, 2015) but all these metabolites come from non-carcinogenic compounds. Thus, BaP exposure monitoring remains the most relevant way for assessing exposure to carcinogenic PAH.

Once BaP has been absorbed into organism, it undergoes metabolic transformation mediated by enzymes of the CYP450 family to form epoxides (Xue and Warshawsky, 2005). These reactive compounds can spontaneously rearrange to form phenols or be catalytically hydrated to form dihydrodiols (Angerer et al., 1997). 3-Hydroxybenzo(*a*)pyrene (3-OHBaP) is one phenol which has been previously proposed as a good surrogate for assessing occupational carcinogenic PAH exposure (Barbeau et al., 2015; Barbeau et al., 2014; Forster et al., 2008; Gendre et al., 2004; Lafontaine et al., 2006; Lafontaine et al., 2004). However, this biomarker is not suitable for low exposure as environmental exposures in which case urinary concentrations were systematically undetected (Health-Canada, 2015; Leroyer et al., 2010). Moreover, being spontaneously produced from reactive epoxide, 3-OHBaP represents the detoxification pathway of BaP metabolism. Conversely, BaP-7,8-dihydrodiol produced by epoxide hydrolase can be oxidized by enzymes of the CYP450 family to form BaP-7,8-diol-9,10-epoxide (BPDE), the ultimate carcinogenic metabolite of BaP responsible for DNA adduct formation (Xue and Warshawsky, 2005). The majority of this reactive metabolite is hydrolyzed producing four different isomers of 7,8,9,10-tetrahydroxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene (7,8,9,10-OHBaP) (Geacintov et al., 1980; Yang et al., 1977). The *trans-anti*-7,8,9,10-OHBaP reached 18% of the dose while *cis-anti*-7,8,9,10-OHBaP, *cis-syn*-7,8,9,10-OHBaP and *trans-syn*-7,8,9,10-OHBaP represented 1.2%, 0.8% and 0.4%, after BaP incubation of human hepatocytes (Zhong et al., 2011). The dextrogyre enantiomer (+)*trans-anti*-7,8,9,10-OHBaP is a product of BPDE while the levogyre enantiomer (−)*trans-anti*-7,8,9,10-OHBaP comes from non-carcinogenic BaP-7,8-epoxide-9,10-diol (reverse-BPDE). The chiral separation of these enantiomers is unnecessary because > 70% of racemic (±)*trans-anti*-7,8,9,10-OHBaP analyzed in human urine is originated from BPDE (Hecht et al., 2010; Hecht and Hochalter, 2014). Thus, urinary racemic (±)*trans-anti*-7,8,9,10-OHBaP seems to be a relevant PAH exposure biomarker linked to the BaP proportion involved into carcinogenic BPDE pathway.

Few studies have performed 7,8,9,10-OHBaP analysis because a highly sensitive and specific analytical method is necessary to quantify the small amounts of this metabolite. An interesting method has been recently published to analyze this biomarker in hair (Grova et al., 2016). Concerning urinary 7,8,9,10-OHBaP analysis in human, the use of gas chromatography (GC) coupled with tandem mass spectrometry allowed analytical sensitivity improvement of two orders of magnitude in comparison to single mass spectrometry (Hecht et al., 2010; Simpson et al., 2000). However, pre-analytical steps remained complex with two successive solid phase extraction (SPE) or liquid-liquid extraction and several evaporations to dryness responsible for internal standard yield below 50% (Zhong et al., 2011; Hilton et al., 2017). Furthermore, there is a lack of knowledge about the relation between external BaP exposure and urinary 7,8,9,10-OHBaP levels as well as about its urinary excretion both for non-occupationally and occupationally exposed subjects, preventing a broad use of this promising carcinogenic PAH exposure biomarker.

The aim of this study was thus to analyze urinary (±)*trans-anti*-7,8,9,10-OHBaP among non-occupationally exposed subjects and workers involved in electrodes production using a highly sensitive routine analytical method with only one SPE pre-analytical step. To allow the use of this new biomarker for carcinogenic PAH exposure assessment, diuresis correction mode, urinary half-life and sampling time were determined. Furthermore, the relations between urinary 7,8,9,10-OHBaP concentrations and tobacco consumption, atmospheric BaP levels and urinary 3-OHBaP were studied.

2. Materials and methods

2.1. Chemicals and reagents

All aqueous solutions were prepared with purified water using the Milli-Q treatment system (Merck Millipore, Germany). Acetate buffer AVS titrinorm solution (pH = 4.66), formic acid (HCOOH) AnalaR Normapur solution (99–100%), and acetonitrile (ACN) HiperSolv Chromanorm (≥ 99.9%) were obtained from VWR chemicals (USA). Methanol (MeOH) Chromasolv V (≥ 99.9%) and pyridine solution (99.8%) came from Sigma-Aldrich (USA). Ammonium hydroxide (NH4OH) solution (25%) Suprapur was obtained from Merck, and trimethylsilyl 2,2,2-trifluoro-*N*-(trimethylsilyl)acetimidate + chlorotrimethylsilane (BSTFA + TMCS) (99:1) solution from Supelco (USA). β-Glucuronidase arylsulfatase solution was supplied by Roche Diagnostics GmbH (Germany). Methane (> 99.9%) and argon (> 99.9%) were obtained from Air Liquide (France). All 7,8,9,10-OHBaP and ^{13}C6-7,8,9,10-OHBaP standard solutions were prepared in ACN using calibration standard of 7,8,9,10-OHBaP (≥ 99.9%) purchased from MRIGlobal (USA) and solution of internal standard ^{13}C6-7,8,9,10-OHBaP (99%) at 100 μg/mL in MeOH from LGC Standards (France).

2.2. Apparatus

The SPE system was an ASPEC GX-271 (Gilson, France). The SPE cartridges were Oasis MAX 150 mg with a capacity of 6 mL (Waters, USA). Evaporation of eluates was performed with a Labconco laboratory concentrator (USA) coupled with a Vacuubrand CVC 3000 pump (Germany). Liquid chromatography analyses were performed by a Waters (USA) 2695 separation module using a chromatographic column Alltech Alltima C18 (250 mm × 4.6 mm, 5 μm) from Grace (USA) coupled with a Waters 2475 multi-wavelength fluorescence detector (HPLC-FLD). GC analyses were performed by an Agilent (USA) GC 7890B with a large volume multimode injector, using a chromatographic column DB17-MS (0.25 mm × 30 m, 0.25 μm) from Agilent coupled with an Agilent tandem mass spectrometer 7000C with negative chemical ionization source (GC-NICI-MS/MS).

2.3. Urinary 7,8,9,10-OHBaP analytical method

2.3.1. Pre-analytical procedure

10 mL aliquot of urine was hydrolyzed at 37 °C overnight after addition of 5 mL of acetate buffer and 20 μL of β-glucuronidase/arylsulfatase. This enzymatic hydrolysis step was not used when urinary unconjugated 7,8,9,10-OHBaP was analyzed. After adding 50 μL of ^{13}C6-7,8,9,10-OHBaP solution (0.1 ng/mL) and 4 mL of MeOH, the mixture was centrifuged for 10 min at 4000 rpm. For extraction step, the SPE cartridge was conditioned with 5 mL of MeOH and 10 mL of H_2O before three loadings of 6.33 mL aliquots of sample. Purification was performed by washing the cartridge successively with 5 mL of MeOH/HCOOH 2% (20/80), 5 mL of MeOH/H_2O (20/80), 5 mL of MeOH/NH_4OH 2% (20/80) and 5 mL of MeOH/H_2O (20/80). For elution, 5 mL of MeOH was required and the methanol extract was