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Measurement of urinary Benzo[*a*]pyrene tetrols and their relationship to other polycyclic aromatic hydrocarbon metabolites and cotinine in humans

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HIGHLIGHTS

- Other B[*a*]P sources than tobacco smoke are significant among smokers and non-smokers.
- Difference in urine level by smoking status is greater for PYE and smaller PAH metabolites than B[*a*]P.
- Excretion profile for B[*a*]P and other PAH metabolites are similar after a dietary exposure.
- Validated measurement method for B[*a*]P in urine is presented.

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ABSTRACT

Biomonitoring of exposure to polycyclic aromatic hydrocarbons (PAHs) typically uses measurement of metabolites of PAHs with four or less aromatic rings, such as 1-hydroxypyrene, even though interest may be in exposure to larger and carcinogenic PAHs, such as benzo[*a*]pyrene (B[*a*]P). An improved procedure for measuring two tetrol metabolites of B[*a*]P has been developed. Using 2 mL urine, the method includes enzymatic deconjugation of the tetrol conjugates, liquid-liquid extraction, activated carbon solid phase extraction (SPE) and Strata-X SPE, and gas chromatography–electron capture negative ionization –tandem mass spectrometric determination. Limits of detection were 0.026 pg/mL (benzo[*a*]pyrene-*r*-7,t-8,t-9,c-10-tetrahydrotetrol, BPT I-1) and 0.090 pg/mL (benzo[*a*]pyrene-*r*-7,t-8,c-9,c-10-tetrahydrotetrol, BPT II-1). We quantified BPT I-1 and BPT II-1 in urine from a volunteer who consumed one meal containing high levels of PAHs (barbequed chicken). We also measured urinary concentrations of BPT I-1 and BPT II-1 in smokers and nonsmokers, and compared these concentrations with those of monohydroxy PAHs (OH-PAHs) and cotinine. Urinary elimination of BPT I-1 and BPT II-1 as a function of time after dietary exposure was similar to that observed previously for OH-PAHs. While the median BPT I-1 concentration in smokers' urine (0.069 pg/mL) significantly differs from nonsmokers (0.043 pg/mL), BPT I-1 is only weakly correlated with cotinine. The urinary concentration of BPT I-1 shows a weaker relationship to tobacco smoke than metabolites of smaller PAHs, suggesting that other routes of exposure such as for example dietary routes may be of larger quantitative importance.

1. Introduction

Smoking, diet, and combustion processes are common sources of exposure to polycyclic aromatic hydrocarbons (PAHs) in the general population (EFSA, 2008). While PAHs, as a class, are recognized as hazardous, individual PAHs possess different degrees of toxicity and carcinogenicity (EPA, 2010). For example, benzo[*a*]pyrene (B[*a*]P) is a human carcinogen, while phenanthrene and pyrene are not classified as human carcinogens (IARC, 2002, 2010). Due to the absence of reliable methods for assessing B[*a*]P exposure in humans, indirect methods have been used, such as urinary 1-hydroxypyrene, a metabolite of pyrene that has been shown to correlate with external exposure to PAHs, including B[*a*]P (Brandt and Watson, 2003). However, the relative proportion of B[*a*]P and pyrene differs from source to source (Dennis et al., 1991; Khalili et al., 1995; Lee et al., 2011), adding to the uncertainty in the

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estimated exposure of B[*a*]P when only based on 1-hydroxypyrene measurements. While B[*a*]P exposure from tobacco smoke can be estimated based on knowledge of the smoke intake and composition of the smoke (Ding et al., 2012), such estimates are associated with uncertainty because smoke chemistry varies across individual tobacco products and individual smokers' puff frequencies and durations (St Charles et al., 2010). For these reasons, the use of specific B[*a*]P human exposure biomarkers may be preferable for exposure assessment, particularly when evaluating relative sources of exposure.

B[*a*]P is oxidized by cytochrome P450 to yield several epoxides (two are shown in Fig. 1). The epoxides can rearrange to form monohydroxy B[*a*]P, such as 3-hydroxybenzo[*a*]pyrene (3-OH-B[*a*]P). 3-OH-B[*a*]P has been proposed as a biomarker, but shows a low rate of detection (Lafontaine et al., 2006) or requires large (50 mL) sample volume (Yao et al., 2014). Alternatively, the epoxide may be hydrolyzed to a diol by an epoxide hydrolase (EH), as is shown for the 7,8-epoxide in Fig. 1. The 7,8-diol is further oxidized to the diolepoxide (Conney, 1982), which is hydrolyzed to a tetrol. Methods such as ELISA or ^{32}P-postlabeling, intended to measure B[*a*]P adducts, possess adequate sensitivity but lack specificity as they may also respond to compounds other than the B[*a*]P metabolites (Gyorffy et al., 2008). Quantitative methods with structural specificity for determining B[*a*]P metabolites in blood (Pastorelli et al., 1996; Melikian et al., 1997; Ozbal et al., 2000) and urine (Simpson et al., 2000; Lafontaine et al., 2006) have been reported. Methods to measure adducts of B[*a*]P to DNA or proteins have shown low levels of detection in nonsmokers (Boysen and Hecht, 2003). Ragin et al. (2008) measured BPT I-1 and BPT II-1 individually in human hemoglobin, with BPT II-1 present at levels between 4% and 63% of the BPT I-1 in the same sample. Methods for determining B[*a*]P metabolites in urine have also shown low detection rates for nonsmokers until a method was reported (Zhong et al., 2011) for measuring benzo[*a*]pyrene-*r*-7,t-8,t-9,c-10-tetrahydrotetrol (BPT I-1) in urine. The method uses two solid phase extraction (SPE) steps and gas chromatography – electron capture negative ionization – tandem mass spectrometry (GC-ECNI-MS/MS). This method was reported to give 100% detection in nonsmokers, but depended on a phenylboronate SPE cartridge, which subsequently has not retained BPT I-1 in our hands. This change in performance was also noted in a revised method which provides results for specific B[*a*]P tetrol enantiomers (Hecht and Hochalter, 2014). Both methods detected BPT I-1 in all urine samples analyzed and showed BPT I-1 to be two times higher in smokers than nonsmokers. Neither of these methods reports values for BPT II-1.

While 1-hydroxypyrene is commonly used to estimate exposure to PAHs, this has largely been because of the relative consistency of the composition of PAHs in industrial settings (Hansen et al., 2008). The reliability of 1-hydroxypyrene as a marker in association with the more carcinogenic PAHs, such as B[*a*]P, has yet to be demonstrated in non-occupationally exposed persons, particularly in any attempt to show relative contributions of B[*a*]P from various sources.

We developed a method for measuring BPT I-1 and benzo[*a*]pyrene-*r*-7,t-8,c-9,c-10-tetrahydrotetrol (BPT II-1) in urine. We tested the usefulness of BPT I-1 and BPT II-1 as exposure biomarkers by examining their urinary concentrations in samples from a volunteer who consumed one meal containing high levels of PAHs (barbequed chicken). We also measured concentrations of BPT I-1 and BPT II-1 in commercially obtained smokers and nonsmokers urine and compared these concentrations with those of other urinary PAH biomarkers and cotinine.

2. Experimental procedures

2.1. Materials and standards

All reagents and solvents were of pesticide or equivalent grade. PAH tetrols were obtained from the MRI Global Chemical Carcinogen Repository (Kansas City, MO, USA). $^{13}C_{12}$–decachlorobiphenyl ($^{13}C_{12}$-PCB209) and $^{13}C_6$ labeled BPT I-1 and II-1 were obtained from Cambridge Isotope Laboratory (Andover, MA). All neat standards had purity at or above 99%. β-Glucuronidase type H-1 with sulfatase activity, from *Helix pomatia,* was obtained from Sigma-Aldrich (St. Louis, MO, USA). ENVI-Carb cartridges (0.5 g, 6 mL) were obtained from Supelco (Bellefonte, PA, USA) and Strata-X cartridges (200 mg, 6 mL) were obtained from Phenomenex (Torrance, CA, USA). Standard reference materials SRM 3672 (smokers urine) and SRM 3673 (nonsmokers urine), used as quality control (QC) materials, were obtained from the U.S. National Institute of Standards and Technology (Gaithersburg, MD, USA).

First morning void urine samples from 30 smokers and 30 nonsmokers were purchased from Bioreclamation (Westbury, NY, USA). Smoking status was self-reported at the time of sample collection and was further confirmed by cotinine measurement at the Centers for Disease Control and Prevention (CDC) using an LC-MS/MS method (Wei et al., 2014). Nonsmoker samples with a continine urinalry level above 100 ng/mL were excluded (n = 4), as this level of cotine is expected only in smokers (Haufroid and Lison, 1998).

The collection of urine specimens used to evaluate the excretion profile of BPT I-1 and BPT II-1 after a controlled dietary exposure was described in detail elsewhere (Li et al., 2012). In brief, non-

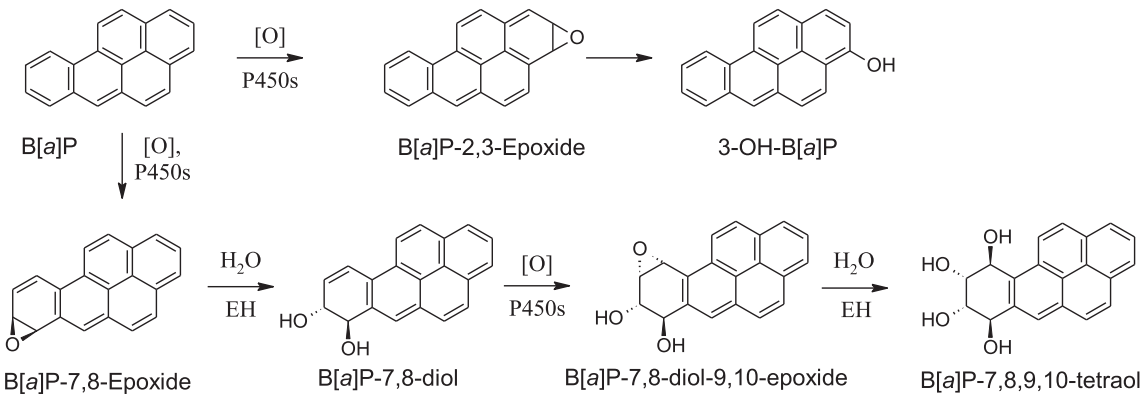

Fig. 1. Pathways to benzo[*a*]pyrene metabolites. The metabolites shown, 3-OH-B[*a*]P and BPT I-1, have been used as biomarkers (Lafontaine et al., 2006; Zhong et al., 2011).