OCCURRENCE OF POLYCYCLIC AROMATIC HYDROCARBON METABOLITES IN URINE OF PORTUGUESE FIREFIGHTERS

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ABSTRACT

The present work estimates occupational exposure of healthy and non-smoking Portuguese firefighters to polycyclic aromatic hydrocarbons (PAHs) through the analysis of four urinary metabolites (OH-PAHs): 1-hydroxyacenaphthene, 1-hydroxynaphthalene, 1-hydroxypyrene (PAH biomarker of exposure), and 3-hydroxybenzo[a]pyrene (PAH biomarker of carcinogenicity). Firemen from several Portuguese corporations were asked to provide urine samples during the winter period (without exposition to fires; pre-fire season) and during the summer season of 2014 after fires fighting. The selected OH-PAHs were extracted from urine samples by solid-phase extraction and analyzed by high-performance liquid chromatography with fluorescence detection. Normalization of the urinary PAH-metabolite levels was achieved by analyzing the creatinine concentrations. 1-hydroxynaphthalene and 1-hydroxyacenaphthene were the most abundant metabolites, followed by 1-hydroxypyrene. The metabolite 3-hydroxybenzo[a]pyrene was not detected. Total OH-PAHs ranged from 0.02 to 4.01 μmol/mol creatinine and between 0.55 to 8.39 μmol/mol creatinine, respectively, for non exposed and exposed firefighters. In general, the detected concentrations of urinary PAH metabolites were higher during the fire season than in the winter season.

Keywords: firefighters, occupational exposure, biomonitoring, urinary PAH-metabolites, liquid chromatography

1. Introduction

Every year, southern European countries such as Portugal, Spain, Italy, and Greece are exposed to severe forest fires which have been reducing forested areas at a remarkable rate. Smoke pollution due to events of forest fires represents potential health risks for the directly affected population but also for personnel involved in fire suppressions. Mortality and morbidity studies of firefighters, although they have produced inconsistent evidence, have raised the possibility of increased risks of cardiovascular and respiratory diseases, cancer of nervous, haematopoietic/lymphatic, respiratory and gastrointestinal systems, probably due to exposure to smoke components [1-2]. Smoke from fires is a complex mixture of gas–, liquid–, and solid–phase chemicals, many of them being known or potential/possible carcinogens to humans [3]. In that regard, polycyclic aromatic hydrocarbons (PAHs) are among the most important air pollutants. U.S. EPA considers 16 PAHs as priority pollutants, some being referred to as persistent organic pollutants and endocrine disrupting chemicals [4]; benzo[a]pyrene is the only PAHs classified as carcinogenic to humans [5]. PAHs are a class of organic chemical compounds that are formed during pyrolysis or incomplete combustion of organic matter.

Humans are exposed to PAHs from air, water and food [6-8]. However, PAHs monitoring is complicated by mixed aerosol/vapour composition of airborne compounds and by absorption of these chemicals from inhalation and dermal contact. For these reasons, investigators have turned to biological monitoring to assess exposures to PAHs. The use of urinary biomarkers of exposure (OH-PAHs) constitutes an effective tool to assess total exposure to PAHs. About 90% of total urinary excretion of pyrene is in the form of 1-hydroxypyrene (1OHPy) [9], making this metabolite the most widely used biological indicator of internal dose of exposure to PAH (PAH biomarker) [10]. 3-hydroxybenzo[a]pyrene (3OHB[a]P) is the most common benzo[a]pyrene metabolite and is more representative of PAHs carcinogenic risk than 1OHPy, thus it has been proposed as PAH carcinogenic biomarker [11]. Naphthalene (possible human carcinogen, [12]) is the most volatile PAH and is eliminated from the human body mainly as 1- or 2-naphthol conjugates [13]. The present study aims to evaluate occupational exposure of Portuguese firefighters to PAHs focusing on the most relevant urinary metabolites: 1-hydroxyacenaphthene, 1-hydroxynaphthalene, 1OHPy and 3OHB[a]P.

2. Experimental

2.1. Sample collection

The urinary samples were collected from twenty healthy and non-smoking firemen in two different Portuguese corporations located in the district of Bragança (North of Portugal). The study subjects were asked to provide urine samples during the winter period (without exposure to fires; pre-fire season) and in the summer season of 2014 after firefighting activities. Urine samples were collected within 8 hours after fire exposure. In addition, the firemen also filled a structured questionnaire in order to characterize the fire incident and to identify other potential exposure routes to PAHs.

2.2. Extraction of oh-pahs

Briefly, an aliquot of 10 mL urine was adjusted to pH 5.0 with 0.5 mol/L HCl and buffered with 20 mL of 0.1 M acetate [14]. After the addition of 20 µL of 1.0 g/L tert-butylhydroquinone and 80 µL of β-D-glucuronidase/arylsulfatase (EC 3.2.1.31/EC3.1.6.1; 5.5/2.6 U mL^{-1}) from Helix pomatia (Roche Diagnostics, Indianapolis, USA) urines were purged under nitrogen flow during 30 min and incubated at 37 ºC for 120 minutes, under constant stirring and in the absent of light. Sep-Pak® Light Plus C18 cartridges (Waters, Portugal) were activated with 5 mL methanol and 10 mL of water. The hydrolyzed urine samples were loaded into the activated C18 cartridges and sequentially washed with 10 mL of water and 10 mL of 20% methanol in water. The cartridges were completely dried under vigorous air flow and manually eluted with 20 mL of methanol/ethyl acetate (10/90). Samples were then evaporated till dryness with a rotary evaporator at room temperature, redissolved in 500 µL of methanol and filtered through a 0.22 µm PTFE syringe filter before chromatographic analysis.

The urinary creatinine levels were quantified according to the Jaffe colorimetric method [15] and were used to normalize the metabolite concentrations.

2.3. Chromatographic analysis

All extracts were analyzed using a Shimadzu LC system (Shimadzu Corporation, Kyoto, Japan) equipped with an LC-20AD pump (high-pressure gradient solvent delivery module equipped with two dual-plunger tandem-flow pumps), DGU-20AS degasser and a fluorescence RF-10AXL (FDL) detector. Separation of the compounds was performed in a C18 column (CC 150/4 Nucleosil 100-5C18 PAH, 150- 4.0 mm; 5 mm particle size; Macherey-Nagel, Duren, Germany) using a mobile phase composed by a mixture of methanol- water. The injected volume was 50.0 µL. Each compound was detected at its optimum excitation/emission wavelength pair: 232/337 nm for 1OHNapt and 1OHAce, 242/388 nm for 1OHPy, and 308/432 nm for 3OHB[a]P. External calibrations with matrix-matched OH-PAHs mixed standards, using at least 5 calibration points, were performed. Calibrations curves were linearly fitted with correlation coefficients always higher than 0.9980 for all compounds. Detection limits in urine samples ranged between 0.0015 µg/L for 1OHPy to 0.31 µg/L for 1OHNapt+1OHAce. Each analysis was performed in triplicate.

3. Results and discussion

Exposure biomarker levels represent the absorbed dose of a chemical, integrated across all microenvironments and routes of exposure. Thus biomarker levels account for factors that modify the relationship between environmental concentrations and dose, including use of personal protective equipment, interindividual differences in absorption, ventilation, exertion, and personal behaviors that modify exposure such as reducing physical activity when smoke levels increase. The most relevant PAH metabolites: 1-hydroxyacenaphthene, 1-hydroxynaphthalene, 1OHPy and 3OHB[a]P were determined in urine, since they have been proposed as biomarkers for smoke exposures. In the majority of PAHs exposure studies, only 1OHPy is used. Combination of several metabolites is a more robust exposure approach than using a single one. Mean total OH-PAHs was 1.74 µmol/mol creatinine (0.02 – 4.01 µmol/mol creatinine) for firefighters during the winter season and 2.54 µmol/mol creatinine (0.55 – 8.39 µmol/mol creatinine) for firefighters during the fire season. 1OHNapt+1OHAce concentrations were 1.94 µmol/mol creatinine (0.50 to 3.99 µmol/mol creatinine) and 2.49 µmol/mol creatinine (0.52 to 8.27 µmol/mol creatinine), respectively, for non-exposed and exposed firefighters (Figure 1). 1OHNapt and 1OHAce were detected at much higher concentrations than 1OHPy (~ 40 to 45 times), contributing with more than 85% for the total urinary OH-PAH levels. Indeed naphthalene and acenaphthene are composed by two fused aromatic rings, and thus they are mainly present in the gas phase of the air, while PAHs with a higher number of aromatic rings tend to be adsorbed to the particulate phase. Regarding the metabolite of the biomarker of exposure to PAHs, 1OHPy, its levels ranged between 0.04 µmol/mol creatinine (0.02 – 0.09 µmol/mol creatinine) to 0.06 µmol/mol creatinine (0.02 – 0.12 µmol/mol creatinine), respectively, during the winter and fire fighting seasons. 3OHB[a]P, the hydroxyl PAH biomarker of carcinogenicity, was not detected. Regarding the possible health impact, this result should be observed with caution since B[a]P metabolites are predominantly excreted through the faeces due to the high number of aromatic rings [16].

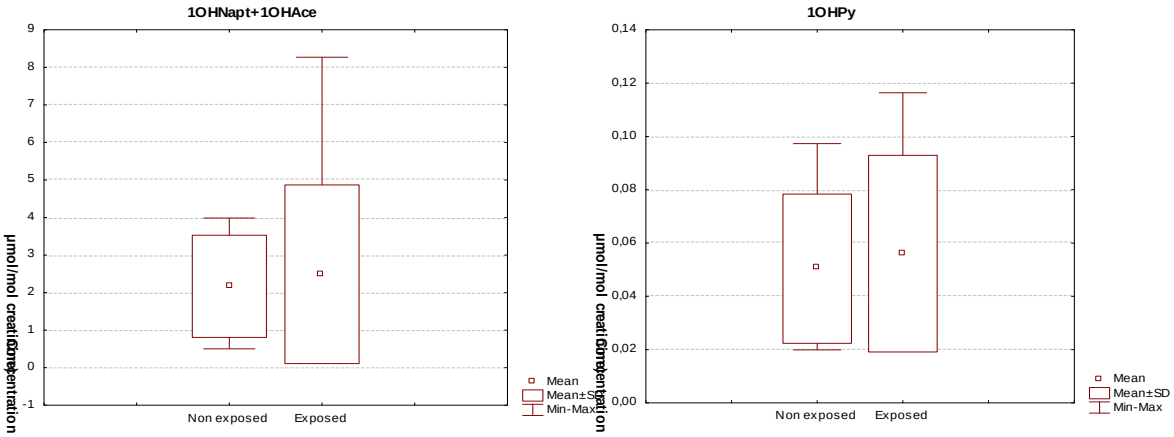

Figure 1: 1OHNapt+1OHAce and 1OHPy concentrations in the urine of non-exposed and exposed Portuguese firefighters.

Concerning the occupational exposure the use of protective equipment, particularly masks, is an important issue for the exposure assessment. Analyzing the data from questionnaires, it may be concluded that all firefighters used a protection suit during fire fighting activities, however very few subjects utilized the respiratory protective equipment. Portuguese professional and volunteer firefighters used only partially or not at all respiratory protection, presumably due to the impression of low smoke intensity (e.g. during the “mop-up” phase of fires), added to the physiological demands and heat stress placed upon users, and difficulty to communicate while wearing a mask. The firemen involved in this study reported an exposure period of 3, 4 and 5 hours in the fire incident. Taking into account the number of hours exposed to fires and the concentrations of OH-PAHs detected, it is possible to observe that higher OH-PAH levels seemed to occur when the duration of exposure to fires increased. Still, this preliminary findings

need to be validated by more comprehensive study. No standard reference or occupational guidelines are available for levels of urinary OH-PAHs. Recently, Jongeneelen [17] proposed a guidance value of 1.0 μmol/mol creatinine for 1OHPy in urine of PAH-exposed workers based on a review of the cross-sectional studies available in the literature. The 1OHPy urinary levels of firefighters were below that threshold while total OH-PAHs exceeded that benchmark even for non-exposed firefighters.

4. Conclusions

The present work contributes to fill a gap regarding the assessment of firefighters occupational exposure through determination of four most common PAH metabolites in urine samples collected during different pre and post fires periods. Overall, the detected concentrations of urinary metabolite were higher in exposed firefighters than in the non-exposed subjects. Considering the relevance of this topic, the present study is currently being extended to a higher number of Portuguese corporations in order to assess exposure in more representative firefighter population. The (chemical and physical) complexity and toxicity of wildfires smoke, as well as physical and emotional stress encountered during firefighting, indicate the need for further studies of firefighters occupational exposure in order to fully comprehend the respective impacts on health. This is especially relevant considering the expected increasing trend of forest fires due to climacteric alterations.

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