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Biomarkers of exposure to carcinogenic PAHs and their relationship with environmental factors

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Abstract

The EXPAH project is a multicentre European study in which biomarkers of exposure, biomarkers of effect, genetic susceptibility and environmental factors were studied in populations exposed to differing levels of carcinogenic polycyclic aromatic hydrocarbons (c-PAHs). We describe here the relationships between the levels of DNA adducts (as biomarkers of exposure), the exposure to air pollution and smoking status. Lymphocyte bulky DNA adducts were significantly correlated with exposure when subjects were classified either by job description or by personal monitor measurements, and both bulky and benzo(*a*)pyrene (B[*a*]P) DNA adducts were also correlated with smoking status. These associations varied across the countries studied (Czech Republic, Slovakia, Bulgaria). Results from a multivariate analysis show that factors mainly contributing to bulky and B[*a*]P DNA adducts are age, smoking habit, country of origin and environmental exposure to c-PAHs. The B[*a*]P DNA adducts were more strongly associated with smoking status than with the environmental exposure to c-PAHs.

Keywords: Benzo[*a*]pyrene; Carcinogenic polycyclic aromatic hydrocarbons; DNA adducts

1. Introduction

Environmental air pollution presents a wide variety of genotoxic compounds, amongst which are the carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) [1]. Epidemiological studies have consistently demonstrated that exposure to PAHs is associated with increases in mortality and/or morbidity from respiratory diseases, cardiovascular diseases and cancer [2–5]. Results from these studies show that populations exposed to environmental pollution have increased levels of several markers of genotoxicity, including bulky DNA adducts (reviewed in [6]), chromosomal aberrations (CA), sister chromatid exchanges (SCE), and ras oncogene overexpression [7,8]. Several biomarkers of exposure to c-PAHs, such as DNA adducts, both total PAH (bulky)

Abbreviations: PAH, polycyclic aromatic hydrocarbon; c-PAHs, carcinogenic PAHs; B[*a*]P, benzo[*a*]pyrene; B[*a*]P DNA adduct, a 'benzo[*a*]pyrene-like' adduct that chromatographed on 2D TLC at the same position as authentic B[*a*]P adduct

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adducts and specific adduct arising from benzo(*a*)pyrene (B[*a*]P) in lymphocyte DNA, have been developed, but their application in population studies has proven to be difficult. Among the problems encountered in such studies are the lack of biomarkers of integrated, chronic exposure, and the lack of accurate assessment of exposure. Another issue is the possible effect modifier of polymorphisms in metabolic genes.

The aim of the present study is to evaluate the relationship between the levels of two different DNA adducts, as biomarkers of exposure to c-PAHs, environmental factors, such as smoking status and occupational exposure to air pollution, and genetic susceptibility.

2. Material and methods

For the present analysis, subjects recruited through the EXPAH project were used ($N = 356$). The EXPAH sample included 105 men from Czech Republic (amongst whom 53 were occupationally exposed to c-PAHs), 106 from Slovak Republic (amongst whom 51 were professionally exposed to c-PAHs) and 145 from Bulgaria (amongst whom 100 were occupationally exposed to c-PAHs). Details on the study design are reported elsewhere [9]. Briefly, each participant completed a questionnaire for demographic, smoking and dietary information. Blood and urine samples were collected at the end of a work shift from each participant for determination of adduct levels, genetic susceptibility, and determination of other biomarkers. Smoking status was defined according to cotinine levels measured in the urine collected at the end of the work shift. Subjects were defined as current smokers when cotinine levels (adjusted by creatinine levels) were greater than 500 ng/mg creatinine.

As biomarkers of exposure to c-PAHs, total PAH (bulky) adducts and the specific adduct arising from B[*a*]P (i.e. adducts that chromatographed on 2D TLC at the same position as authentic B[*a*]P adduct—"like" B[*a*]P-DNA adduct spot) were measured in lymphocyte DNA, using ^{32}P-postlabelling and following protocols described elsewhere [10]. B[*a*]P DNA levels were below the limit of quantitation in the samples from Slovak Republic. All the adduct measurements were performed in a single laboratory.

Gene polymorphisms involved in polycyclic aromatic hydrocarbons metabolism, such as CYP1A1 MspI, CYP1B1 Ile/Val, NAT2 acetylation status, and several gluthathione transferases were determined by a PCR-based method.

2.1. Statistical analysis

Data on bulky DNA adducts and B[*a*]P DNA adducts levels are presented as means and standard deviations (S.D.). A new definition of exposure to c-PAHs was defined according to personal exposure monitoring: subjects with c-PAHs levels lower or equal than 7.55 ng/m^3 (the median value of c-PAHs in the whole sample of non-exposed subjects) were defined as "unexposed", while subjects with levels greater than 7.55 ng/m^3 were defined as "exposed".

Since bulky DNA adducts and B[*a*]P DNA adducts levels differ across countries, both variables were standardized by dividing each value for the average adducts levels of the respective country. When necessary, the adducts levels were log or square root transformed in order to have normally distributed values. Differences of bulky DNA adducts and B[*a*]P DNA adducts levels between groups (namely between countries, according to smoking status, and to exposure to c-PAHs) were tested using *t*-test and analysis of variance (ANOVA). To analyze the independent factors contributing to DNA adducts levels, a multivariate analysis (general linear model, GLM) was performed. The model included country, smoking status, exposure to c-PAHs, age, and genetic susceptibility factors (metabolic gene polymorphisms) as independent factors. Due to the high difference between exposure to c-PAHs status defined by the type of job and measured by the personal monitoring, all the analyses that included these variables were performed using separately both exposure definitions.

Pearson analysis has been use to evaluate the correlation between adducts levels and age. *P* values lower than 0.05 were considered as statistically significant. All the statistical analyses were performed using SAS statistical package (8.1 Version, SAS Institute Inc., Cary, NC).

3. Results

Three hundred and fifty six subjects were included in the present analysis. The mean overall values of bulky DNA adducts and B[*a*]P DNA adducts were 1.06 ± 0.40 and 0.12 ± 0.04 adducts/10^8 nucleotides, with significant differences among countries. Bulky DNA adducts were significantly higher ($p < 0.0001$) in Bulgarian subjects (1.33 ± 0.34 adducts/10^8 nucleotides) than in Slovakian (0.88 ± 0.40 adducts/10^8 nucleotides) or in Czech subjects (0.87 ± 0.26 adducts/10^8 nucleotides). B[*a*]P DNA adducts were only available for Bulgaria and Czech Republic; the values were 0.13 ± 0.04 and 0.11 ± 0.04 adducts/10^8 nucleotides, respectively.

For the whole population, total bulky DNA adducts and B[*a*]P DNA adducts were significantly more abundant in smokers compared to non-smokers ($p = 0.01$ and 0.003, respectively; Table 1), and in the exposed group compared to the non-exposed group using job definition of exposure ($p < 0.0001$ and $p = 0.002$, respectively), while using levels of personal monitor only the total bulky DNA adducts were significantly higher in exposed subjects ($p = 0.007$) (Table 2).

When stratified by country, the association between smoking status and DNA adducts levels was still observed in Czech Republic, where both total bulky DNA adducts and B[*a*]P DNA adducts levels were more