Environmental Research 126 (2013) 98–104

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Polycyclic aromatic hydrocarbon biomarkers and serum markers of inflammation. A positive association that is more evident in men

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ARTICLE INFO

Article history:
Received 15 April 2013
Received in revised form
15 July 2013
Accepted 19 July 2013
Available online 22 August 2013

Keywords:
Polycyclic aromatic hydrocarbons
Inflammation
Serum C-reactive protein
Total white blood cell count
NHANES

ABSTRACT

Background: Polycyclic aromatic hydrocarbons (PAHs) are potent atmospheric pollutants, occurring from anthropogenic and natural sources. Several animal studies have reported a positive association of PAHs with inflammation. However, it is not clear if lower background exposure to PAHs is associated with inflammation in humans, independent of smoking, a major source of PAHs.
Methods: We examined participants from the National Health and Nutrition Examination Survey 2001–2002, 2003–2004, and 2005–2006. Our exposures of interest were eight urinary monohydroxy polycyclic aromatic hydrocarbon biomarkers. Our outcomes were serum markers of inflammation; C-reactive protein (CRP) (≤10 mg/L) and total white blood cell (WBC) count (4000–12,000 cells/μL).
Results: Compared to participants with summed biomarkers of low-molecular weight (LMW) PAHs in the lowest quartile, the multivariable odds ratios (95% confidence interval) of high serum CRP (≥3 mg/L) and high total WBC count (defined as at or above the 95 percentile of total WBC distribution) among participants in the highest exposure quartile were 1.77 (1.13, 2.76) and 1.34 (1.12, 1.60) respectively. Urinary 1-hydroxypyrene, the biomarker of the higher molecular weight pyrene, was positively associated with total WBC count, and to lesser extent with serum CRP. In subsequent analyses, the positive association between LMW PAHs and serum CRP and total WBC count was found to be present within the stratified subgroups, independent of smoking and other potential confounders. The positive association was more evident among adult males when compared to females.
Conclusions: Urinary PAH biomarkers were found to be positively associated with serum CRP and total WBC count independent of smoking and other potential confounders. The association was more evident in men.

1. Introduction

Systemic inflammation is considered a key risk factor for atherosclerosis and subsequent development of cardiovascular disease (CVD) (Tracy, 1998). Several studies have reported a positive association between baseline elevations of C-reactive protein (CRP), a serum inflammatory maker, and future risk of CVD (Ridker et al., 1997; Ridker et al., 1998). Clinical and public health groups have recommended serum CRP levels to be used as a CVD risk stratifying tool (Yeboah, 2012). In addition, elevations in total white blood cells (WBC) count within the normal range (4000–12,000 cells/μL) were found to be independently associated with increased risk of CVD and have been proposed as an alternate serum inflammatory marker (Kannel et al., 1992).

Polycyclic aromatic hydrocarbons (PAHs) are potent atmospheric pollutants composed of fused aromatic rings (Talaska et al., 1996; Angerer et al., 1997; Warshawsky, 1999). PAHs may occur in oil, coal, and tar deposits, and are produced as byproducts of indoor and outdoor fuel burning (Liu et al., 2008; Achten and Hofmann, 2009). PAHs can be also found in contaminated water and in food as a result of food processing, preparation, and cooking (Ramesh et al., 2004; Šimko, 2005). Further, exposure to PAHs is markedly increased by cigarette smoking. Several in-vitro and animal studies have reported a positive association between exposure to PAHs and systemic inflammation (Albert et al., 1977; Penn et al., 1981; Curfs et al., 2005; Jeng et al., 2011). However, it is not clear if the lower background exposure to PAHs is associated with inflammatory effects in humans in the general population.

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http://dx.doi.org/10.1016/j.envres.2013.07.006

In this context, we examined the association of eight urinary biomarkers of PAHs, the monohydroxy-PAHs (OH-PAH), with serum CRP and total WBC count in a nationally representative sample of United States (US) adults. Since exposure to active smoking and second hand cigarettes smoke are major sources of PAHs, we sought to determine if this relationship was independent of serum cotinine, self-reported cigarettes smoking status, and other potential confounders.

Due to the multiple sources of PAHs in the environment, exposure to a single PAH compound is implausible. Metabolism, and consequently health effects of exposure to multiple PAHs were found to be different from that of exposure to an individual PAH compound (Olatubi, 2005). Enzyme competition was evident in the metabolism of PAH mixtures, changing significantly the metabolism patterns from that of individual PAHs (Olatubi, 2005). Therefore in the current study, and similar to analytical strategies employed by previous authors (Xia et al., 2009), we created a summed variable as a measure of cumulative exposure to multiple low molecular weight PAHs simultaneously.

2. Methods

2.1. Study population

The present study is based on merged data from the 2001–2002, 2003–2004 and 2005–2006 National Health and Nutrition Examination Survey (NHANES). Detailed description of NHANES study design and methods are available elsewhere (Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS) 2001). NHANES included a stratified multistage probability sample representative of the civilian non-institutionalized US population. Selection was based on counties, blocks, households and individuals within households, and included oversampling of non-Hispanic Blacks and Mexican Americans in order to provide stable estimates of these groups. Out of 31,509 participants in NHANES 2001–2006, there were 11,512 who were 20–65 years of age. Urinary PAH biomarkers were only measured in a subsample of individuals. The subsample is nationally representative, but with a smaller analytic sample size.

We excluded participants with missing information on serum CRP or with CRP levels >10 mg/L, indicating potential underlying non-cardiovascular causes of inflammation (Pearson et al., 2003). We further excluded participants with missing information on serum cotinine level, or other covariates included in the final CRP model. Similarly, to minimize the confounding effect of infection, only subjects with a WBC count within the normal range (4000–12,000 cells/μL) were included in the final WBC analysis. We also excluded participants with missing information on total WBC count, or other covariates included in the final model.

2.2. Main outcome of interest: serum inflammatory markers

2.2.1. High sensitivity serum C-reactive protein

Serum CRP was measured using latex-enhanced nephelometry. Details of the laboratory collection, processing, and analysis are available in the laboratory procedures manual (Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS) 2001). High CRP level was defined as values ≥3 mg/dL, consistent with American Heart Association/Centers for Disease Control & Prevention (AHA/CDC) guidelines for identifying subjects with high risk of CVD (Pearson et al., 2003).

2.2.2. Total white blood cell count within normal values

The methods used to derive WBC count are based on the Beckman Coulter method of counting. High WBC count was defined as values at or above the 95th percentile of the total WBC count distribution, consistent with previous studies examining the association between total WBC count within normal ranges and CVD risk (Kannel, 1992; Twig et al., 2012).

2.3. Main exposure: urinary levels of monohydroxy-PAH

Urine specimens collected during the clinical exam portion of the survey were processed, stored, and shipped to the Division of Environmental Health Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention for analysis. The specific analytes measured in this study were monohydroxy-PAH (OH-PAH). By evaluating these analytes in urine, a measurement of the body burden from PAH exposure is obtained (Castano-Vinyals et al., 2004). The procedure involves enzymatic hydrolysis of urine, extraction, derivatization and analysis using capillary gas chromatography combined with high resolution mass spectrometry (GC-HRMS). Detailed specimen collection and processing instructions are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM).

Seven LMW PAH urinary biomarkers, naphthalene biomarkers; 1-hydroxynaphthalene, 2-hydroxynaphthalene, fluorene biomarkers; 2-hydroxyfluorene, 3-hydroxyfluorene, phenanthrene biomarkers; 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene and 1-hydroxypyrene, the biomarker of the higher molecular weight PAH pyrene, were consistently available in NHANES 2001–2006. All analytes were measured in the same unit; ng/L. Urinary OH-PAH were corrected for urinary creatinine concentration, a urinary marker of kidney function to adjust for urinary dilution (Barr et al., 2005). Urinary levels of OH-PAH (ng/L) were divided by urinary creatinine level (mg/dL) multiplied by 0.01; [(ng/L)/(mg/dL × 0.01)] and expressed as nanogram per gram of creatinine (ng/g creatinine).

2.4. Exposure measurements

Information on age, gender, race/ethnicity, alcohol intake, income, diabetes and cigarette smoking were obtained from a standardized questionnaire during a home interview. Alcohol consumption was categorized into none and alcohol drinker. Income-poverty ratio (income/poverty guideline) was used as a measure of the socioeconomic status. The Department of Health and Human Services' poverty guidelines were used as the poverty measure to calculate this ratio. Cigarettes smoking status was categorized into never smokers (smoked <100 cigarettes during their lifetime), former smokers (smoked ≥100 cigarettes during their lifetime and currently not smoking), current smokers (smoked ≥100 cigarettes during their lifetime and currently smoking). Information on anthropometric, physical and laboratory components were obtained during the medical examination center examination. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Seated blood pressure was measured using a mercury sphygmomanometer according to the American Heart Association and recommendations (Chaturvedi, 2004). Up to 3 measurements were averaged for blood pressure.

2.5. Statistical analysis

Urinary levels of OH-PAH were analyzed both as continuous as well as categorical variables. For analysis as continuous variables, urinary OH-PAH levels were log-transformed as a result of their skewed distribution. Weighted Pearson correlation coefficients between individual OH-PAH were calculated to evaluate the correlations between pairwise combinations of all eight urinary metabolites. We created a summed LMW PAH biomarkers variable by summing urinary levels of metabolites of the low molecular weight PAHs (naphthalene, fluorene and phenanthrene).

We ran linear regression models to calculate the multivariable change and 95% confidence interval (CI) in serum CRP and total WBC count with increasing individual and additive urinary OH-PAH levels. In addition, we ran logistic regression models to calculate the multivariable odds ratio (OR) and 95% CI of high serum CRP (≥3 mg/L) and total WBC count in the 95th percentile, for each higher urinary OH-PAH quartile by using the lowest quartile as the referent. Variables were included in the model if they satisfied a plausible association with the main exposure/outcome. In addition, we used stepwise adjustment to identify the variables that could predict changes in serum CRP as well as total WBC count. Inclusion and retention of variables were allowed at a 10% change of odds ratio after adjusting for the potential confounder. Accordingly, final models were adjusted for age (years), sex (men, women), ethnicity (non-Hispanic White, non-Hispanic Black, all others), poverty-income ratio (%), alcohol drinking (yes/no), diabetes (absent/present), BMI (normal, overweight, obese), total cholesterol (mg/dL), serum cotinine (ng/mL) and systolic blood pressure (mm Hg).

To further ensure that the association is parallel for subgroups, we performed subgroup analyses by gender, race/ethnicity, BMI and self-reported cigarettes smoking categories. Sample weights that account for the unequal probabilities of selection, oversampling, and nonresponse in the NHANES survey were applied for all analyses. Analyses were conducted using SAS (version 9.3, SAS Institute, Cary, NC) software. Standard errors were estimated using the Taylor series linearization method.

3. Results

Table 1 presents the baseline characteristics of the study population with CRP levels <10 mg/L. The study population was primarily non-Hispanic white (72.9%). Approximately one-half (50.5%) were never smokers, and the remainders were former smokers (21.7%) and current cigarettes smokers (27.8%). The arithmetic mean of serum cotinine level was 74.2 ng/mL.