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The effects of heavy metals and their interactions with polycyclic aromatic hydrocarbons on the oxidative stress among coke-oven workers

Tian Wang [a], Wei Feng [a], Dan Kuang [a], Qifei Deng [a], Wangzhen Zhang [b], Suhan Wang [a], Meian He [a], Xiaomin Zhang [a], Tangchun Wu [a], Huan Guo [a,*]

[a] Department of Occupational and Environmental Health, State Key Laboratory of Environmental Health (Incubating), School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
[b] Institute of Industrial Health, Wuhan Iron & Steel (Group) Corporation, Wuhan 430070, China.

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ABSTRACT

Heavy metals and polycyclic aromatic hydrocarbons (PAHs) are predominate toxic constituents of particulate air pollution that may be related to the increased risk of cardiopulmonary events. We aim to investigate the effects of the toxic heavy metals (arsenic, As; cadmium, Cd; chromium, Cr; nickel, Ni; and lead, Pb), and their interactions with PAHs on oxidative stress among coke-oven workers. A total of 1333 male workers were recruited in this study. We determined their urinary levels of As, Cd, Cr, Ni, Pb, twelve PAH metabolites, 8-hydroxydeoxyguanosine (8-OHdG), and 8-iso-prostaglandin-F2α (8-iso-PGF2α). Multivariate linear regression models were used to analyze the effects of these metals and their interactions with PAHs on 8-OHdG and 8-iso-PGF2α levels. It was found that only urinary As and Ni showed marginal or significant positive linear dose-dependent effects on 8-OHdG in this study population, especially among smokers ($\beta=0.103$, $P=0.073$ and $\beta=0.110$, $P=0.002$, respectively). After stratifying all participants by the quartiles of ΣOH-PAH, all five metals showed linear association with 8-OHdG in the highest quartile subgroup (Q4) of ΣOH-PAHs. However, these five urinary metals showed significantly consistent linear associations with 8-iso-PGF2α in all subjects and each stratum. Urinary ΣOH-PAHs can significant modify the effects of heavy metals on oxidative stress, while co-exposure to both high levels of ΣOH-PAHs and heavy metals render the workers with highest 8-OHdG and 8-iso-PGF2α (all $P_{interaction} \leq 0.005$). This study showed evidence on the interaction effects of heavy metals and PAHs on increasing the oxidative stress, and these results warrant further investigation in more longitudinal studies.

1. Introduction

Particulate air pollution has been reported to be associated with the increased risk of cardiovascular diseases as well as lung cancer morbidity and mortality and is of great public health threat (Madrigano et al., 2013; Turner et al., 2011). Particulate matters (PM) are composed of various biologically active constituents including polycyclic aromatic hydrocarbons (PAHs) and heavy metals (Bae et al., 2010; Wei et al., 2009). Although many studies have consistently shown the damaging health effects of exposure to PAHs and heavy metals separately (Kim et al., 2013; Rota et al., 2014; Wong et al., 2005), there is particular concern that co-exposure to PAHs and toxic metals may result in additive, suppressive, or synergistic toxic biological effects (Haguenoer et al., 1996; Huang et al., 2013; Vakharia et al., 2001). However, little is known about the interaction effects of these two types of pollutants, and data on the underlying mechanisms of such effects remain scarce currently.

Coke-oven workers are a typical population generally exposed to a mixture of contaminants, such as polycyclic aromatic hydrocarbons (PAHs), which are the major pollutants released from incomplete combustion of coal (Mu et al., 2012; Passant et al., 2002). Coal also contains trace amounts of metallic elements released during carbonization. These elements often include toxic heavy metals, especially arsenic (As), cadmium (Cd), chromium (Cr), nickel (Ni), and lead (Pb) (Mu et al., 2012; Passant et al., 2002). The carcinogenic potential of these heavy metals has been widely

Abbreviations: PAHs, polycyclic aromatic hydrocarbons; ROS, reactive oxygen species; 8-OHdG, 8-hydroxydeoxyguanosine; 8-iso-PGF2α, 8-iso-prostaglandin-F2α; ΣOH-PAHs, total concentration of urinary monohydroxy PAHs; LOD, limits of detection.

* Corresponding author. Fax: +86 27 83657765.
E-mail address: ghuan5011@hust.edu.cn (H. Guo).

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studied in humans and experimental animals ('t Mannetje et al., 2011; Boffetta et al., 2011; Huang et al., 2004; Joseph, 2009; O'Brien et al., 2003; Oller, 2002; Verougstraete et al., 2003). The unifying factor in determining toxicity and carcinogenicity for all these metals is the generation of free radicals and oxidative stress, which can then cause oxidative damage to DNA and enhanced lipid peroxidation (Jeng et al., 2011; Valko et al., 2005).

Indeed, present evidence indicates that 8-hydroxy-deoxyguanosine (8-OHdG) in urine is a widely accepted critical biomarker of oxidative DNA damage, and may be a risk factor for cancer and atherosclerosis (Wu et al., 2004). Also, 8-iso-prostaglandin-F2α (8-iso-PGF2α), an isoprostane produced by the non-enzymatic peroxidation of arachidonic acid in membrane phospholipids, is a reliable biomarker for the identification of subjects with enhanced rates of lipid peroxidation (Basu, 2010), which ultimately produces damage to proteins and DNA and is associated with carcinogenesis and coronary heart disease (Schwedhelm et al., 2004; Wang et al., 2006). Thus, determination of urinary levels of 8-OHdG and 8-iso-PGF2α can reflect the general oxidative stress levels of the body. The urinary concentrations of heavy metals and PAH metabolites have been used as good biomarkers indicating individual's short-term or long-term internal exposure dose and have been used in many epidemiological studies (Aguilera et al., 2010; Aitio et al., 2007; Kuang et al., 2013). Our previous study has reported that the total concentrations of urinary PAH metabolites showed significant dose-increased relationships with 8-OHdG and 8-iso-PGF2α among coke-oven workers (Kuang et al., 2013). But little data regarding the potential harmful impacts of heavy metals on this category of workers is available.

In this study, we conducted a cross-sectional study of 1333 male coke-oven workers in Wuhan, China, attempted to evaluate the exposure degree of toxic heavy metals, namely; As, Cd, Cr, Ni, and Pb, and to explore the effects of these heavy metals and their interactions with PAHs on oxidative stress levels. We determined the urinary levels of heavy metals including As, Cd, Cr, Ni and Pb, and twelve PAH metabolites including 1-hydroxynaphthalene (1-OHNa), 2-OHNa, 2-hydroxyfluorene (2-OHFlu), 9-OHFlu, 1-hydroxyphenanthrene (1-OHPh), 2-OHPh, 3-OHPh, 4-OHPh, 9-OHPh, 1-OHP, 6-hydroxychrysene (6-OHChr), and 3-hydroxybenzo[a]pyrene (3-OHBaP), and then evaluated the oxidative damage levels to DNA (urinary 8-OHdG) and lipid peroxidation (8-iso-PGF2α) in the study population.

2. Materials and methods

2.1. Study population

The study population consisted of 1333 healthy male workers who had worked in a coke oven plant in Wuhan (Hubei, China) for at least one year. All subjects gave informed consent to participate in this research. A questionnaire was administered to all subjects to obtain information on their demographics: health status, body weight, height, smoking status, alcohol consumption, occupational history, and employment time, after obtaining their written informed consent to participate in this study. Subjects who had smoked >1 cigarette per day for at least 1 year over their lifetime were defined as smokers; otherwise, subjects were defined as non-smokers. The questions on alcohol included the number of days per week each alcoholic beverage (i.e. white wine, red wine, beer) was consumed, the daily number and volume of drinks, and the duration of the habit. Those who had drunk alcohol at least one time in every month for more than 6 month were defined as alcohol drinkers; otherwise, subjects were defined as non-alcohol drinkers. A spot morning 20-mL urine sample was collected from each subject and stored at $-80\,^{\circ}C$ before being analyzed. The study protocol was approved by the Ethics and Human Subject Committee of Tongji Medical College.

2.2. Determination of urinary heavy metals

The urinary concentrations of metals were analyzed by inductively coupled plasma-mass spectrometer (Agilent 7700Xseries ICP-MS; Agilent Technologies, USA) based on the method described by Heitland and Köster with some modifications (Heitland and Köster, 2006). For samples introduction into the ICP, the urine samples were diluted 1/5 (V/V) with ultrapure water and 1.2% HNO_3 (Merck, Darmstadt, Germany). The standard reference material (SRM) 2670 a (Toxic Elements in Urine) and 1640a (Trace Elements in Natural Water) purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) were used as quality control. We estimated the accuracy by comparing the difference between the available certified values and the measured values with their uncertainty according to the calculation method reported by Linsinger et al. (Linsinger, 2005). The measurement results of our method were consistent with the SRM 2670a certified values. The method accuracy for Pb and Cd were verified by SRM 2670a, while Cr, Ni, and As in SRM 2670a were not certified. However, the mean results of As, Cr, and Ni by the assay agreed within 7.5% of the reference values given by NIST. Additionally, spiked pools were used for external calibration. When measuring of the urinary metals, twenty urine samples were set as a batch and the multi-element standards SRM 1640a was re-analyzed after each batch to ensure the normal performance of the instrument. If the detected values of SRM 1640a were not in agreement with their actual concentrations, the instrument was recalibrated and twenty urine samples were re-tested again. The limits of detection (LOD) for these 5 metals were 0.004 μg/L for As, 0.001 μg/L for Cd, 0.009 μg/L for Cr, 0.01 μg/L for Ni, and 0.051 μg/L for Pb. Samples with concentrations below LOD were set to LOD/2 for each metal. The concentrations of urinary creatinine were measured according to Jaffe's colorimetric method using automated clinical chemistry analyzer. The concentrations of urinary metals were calibrated by urinary creatinine and defined as μg/mmolcreatinine.

2.3. Determination of urinary PAH metabolites

A total of twelve urinary PAH metabolites, including 1-OHNa, 2-OHNa, 2-OHFlu, 9-OHFlu, 1-OHPh, 2-OHPh, 3-OHPh, 4-OHPh, 9-OHPh, 1-OHP, 6-OHChr, and 3-OHBaP were determined using the Agilent 5975B/6890N GC/MS System (Agilent, Santa Clara, CA). The detailed methods have been described previously (Kuang et al., 2013). Briefly, 3.0 mL urine of each subject was extracted for analyzing the urinary PAH metabolites and each sample was determined in triplicate. The standard materials with purity $\geq 98\%$ were purchased from Sigma-Aldrich (1-OHNa, 2-OHNa, 2-OHFlu, 9-OHFlu, 9-OHPh, 1-OHP, and 3-OHBaP) (Munich, Germany), Dr. Ehrenstorfer (1-, 2-, 3-, and 4-OHPh) (Augsburg, Germany), and AccuStandard (6-OHChr) (New Haven, CT, USA). The identification and quantification of urinary PAH metabolites were based on retention time, mass-to-charge ratio and peak area using a linear regression curve obtained from separate internal standard solutions.

We eventually detected ten urinary PAH metabolites, except for 6-hydroxychrysene and 3-hydroxybenzo[a]pyrene whose concentrations were below LOD. The LOD for the urinary PAH metabolites ranged from 0.1 μg/L to 1.4 μg/L, and the concentrations of those samples below LOD were set to LOD/2. The concentrations of urinary PAH metabolites were also calibrated by urinary creatinine and expressed as μg/mmolcreatinine.