Significant Positive Correlation of Plasma BPDE-Albumin Adducts to Urinary 1-Hydroxypyrene in Coke Oven Workers[1]

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Objective   To investigate the application of BPDE-albumin adducts as monitoring biomarkers for coke oven workers exposed to polycyclic aromatic hydrocarbons (PAHs) and to explore possible relationship between BPDE-albumin adducts and urinary 1-hydroxypyrene (1-OHP) levels in them.   **Methods**   Thirty-seven coke oven workers from a coke plant and 47 controls without the occupational exposure to PAHs were recruited in this study. The levels of plasma BPDE-albumin adducts and urinary 1-OHP were analyzed using high performance liquid chromatography.   **Results**   The median levels of BPDE-albumin adducts (42.10 fmol/mg albumin) and urinary 1-OHP (5.46 μmol/mol creatinine) were significantly higher in coke oven workers than in controls (14.16 fmol/mg albumin, 2.96 μmol/mol creatinine, respectively; $P<0.01$). Multiple logistic regression analysis showed that coke oven workers were at higher risk of having BPDE-albumin adduct levels above 25.30 μmol/mg albumin (OR=1.79, $P<0.01$) and urinary 1-OHP levels above 4.13 μmol/mol creatinine (OR=2.45, $P<0.05$). There was a positive correlation between the levels of BPDE-albumin adducts and urinary 1-OHP in all subjects (r_s=0.349, $P<0.01$).   **Conclusion**   BPDE-albumin adduct is a useful biomarker for monitoring long-term exposure to PAHs, and plasma BPDE-albumin adducts level is significantly correlated to urinary 1-OHP levels in coke oven workers.

Key words: Polycyclic aromatic hydrocarbons; Benzo[*a*]pyrene; Albumin adduct; 1-Hydroxypyrene

INTRODUCTION

Workers at a coke plant are exposed to coke oven emissions containing polycyclic aromatic hydrocarbons (PAHs) which are released into the environment when coal is pyrolysed into coke. Epidemiological studies suggest an etiologic link between carcinogenic PAH exposure and lung cancer in coke oven workers exposed to PAHs, and coke oven workers are at 3- to 7-fold increased risk of developing lung cancer[1-2].

To further understand the health hazards connected with PAH exposure, biomarkers have been monitored in relation to PAH exposure. Different biomarkers have been used to assess the exposure level to genotoxic compounds in general and in the occupational environment. The most commonly used biomarkers of PAH exposure are 1-hydroxypyrene (1-OHP), and PAH-DNA or protein adducts. Urinary PAH metabolites, particularly 1-OHP, have served as a marker of exposure to volatile PAHs mostly in the occupational environment and in smokers. However, urinary biomarkers are eliminated rapidly in the body and reflect only a short-term exposure less than for 24 hours. Thus, it is better to use reliable long-term biomarkers of PAHs in epidemiological studies. One of the long-term biomarkers of PAHs used in occupational studies is protein adduct. Benzo[a]pyrene (B[*a*]P), a well-studied carcinogen in PAH mixtures, has been used as an indicator for carcinogenic PAHs[3-4]. B[*a*]P is metabolically activated to 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo [a]pyrene (BPDE), an ultimate carcinogenic metabolite known to bind covalently to albumin in humans[5].

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Assessment of BPDE-albumin adducts has been proposed as a long-term biomarker of exposure to PAHs, mainly because of their reflecting exposure and metabolic activation over a 1-month life span of albumin. A number of studies have investigated specific BPDE-derived albumin adducts in workers[6-8]. However, there is less evidence for the correlation between BPDE-albumin adducts and urinary 1-OHP levels.

Therefore, the present study aims to assess whether occupational exposure to PAHs results in high levels of BPDE–albumin adducts and urinary 1-OHP and subsequently to study their possible correlation in 37 male workers exposed to PAHs and 47 male unexposed controls.

MATERIALS AND METHODS

Study Subjects and Sample Collection

A total of male 84 subjects were recruited from a steel plant in Taiyuan, North China. The exposed group was comprised of 37 male workers exposed to PAHs regularly who were employed for at least 6 months. Additional 47 male controls were staff members of the offices and hospitals without PAH exposure. The pre-designed questionnaires focused on health status, smoking history, alcohol consumption, and history of occupational exposure of all the individuals. After an informed consent was obtained from all participants, 5 mL venous blood was obtained from each subject in the morning after overnight fasting and urine sample (20 mL each) was collected at the end of the work shift after four successive working days[9]. Plasma was separated by centrifugation. Plasma and urine samples were stored at -80 ℃ for detecting levels of BPDE-albumin adducts and 1-OHP. All samples were analyzed in a blind manner without knowing the subjects' status.

Airborne PAH Monitoring

Four working sites for the exposed group and three working sites for the control group were selected to collect airborne samples three times consecutively, with an average flow rate of 2.0 L/min (Gilian HFS-513 air sampling pumps, Sensidyne, Inc. USA) for 2-5 h (240-600 L/sample). Quantitative chemical analysis of eight carcinogenic PAHs (B[a]P, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, dibenz[a,h]anthracene, benzo[g,h,i]perylene, indeno[1,2,3-cd]pyrene) was performed by high performance liquid chromatography (Waters Corp. Milford, MA) with fluorescence detectors according to the methods 5506 published by the National Institute for Occupational Safety and Health[10].

Determination of Plasma BPDE-albumin Adduct Levels

B[a]P -r-7, t-8, t-9, c-10-tetrahydotetrol (BPDE-I) was purchased from the National Cancer Institute, Chemical Carcinogen Repository (Midwest Research Institute, Kansas City, MO., USA). Plasma albumin was isolated as previously described[11-12] and the albumin levels of each plasma sample were determined with a Bio-Rad (Hercules, CA) protein assay kit. The albumin was adsorbed on preconditioned cartridges. The levels of BPDE bound to plasma albumin were determined by high performance liquid chromatography with fluorescence detector, and the detection of BPDE was carried out on a reverse phase C18 column with 30℃. The mobile phase was methanol and H_2O. The flow rate was 0.8 mL/min. Quantification of BPDE was determined by the peak-area of measurement using the linear regression curve for standard solutions expressed as fmol BPDE equivalents per microgram albumin.

Determination of 1-OHP

Reverse-phase HPLC was used for the quantitative analysis of 1-OHP in urine as previously described[13]. The results were expressed as micromoles per milimole creatinine.

Statistical Analysis

Statistical analysis was done by SPSS 11.5 software (SPSS 11.5. SPSS Inc, Chicago, USA). The frequencies of categorical variables such as smoking and alcohol consumption among groups were compared by Chi-square test. Variables not fitting the normal distribution were compared using non-parametric tests: Mann-Whitney U-tests were used to evaluate the difference in BPDE-albumin adduct and urinary 1-OHP levels between the two groups. Correlations were checked by the Spearman rank-order correlation test. The subjects were stratified into two subgroups according to the median levels of BPDE-albumin adducts and 1-OHP, the association between exposure to PAHs and BPDE-albumin adducts and 1-OHP was investigated using a logistic regression model with adjustment for possible confounders (age, smoking, and alcohol consumption). $P<0.05$ was considered statistically significant.

RESULTS

Main Characteristics of Study Subjects

The basic characteristics of coke oven workers and controls are summarized in Table 1. There were no significant differences in age, duration of

employment, percentages of smokers and alcohol   drinkers between the two groups.

TABLE 1

Main Characteristics of Coke Oven Workers

Variables	Exposed Group	Control Group	P Value
Number	37	47	
Age ($\bar{x} \pm s$)	39.95±1.55	39.57±2.29	0.400 [a]
Duration of employment ($\bar{x} \pm s$)	18.43±4.50	18.49±4.50	0.959 [a]
Current Smokers, n (%)	32 (86.5%)	38 (80.9%)	0.350 [b]
Alcohol Users, n (%)	18 (48.6%)	24 (51.1%)	0.500 [b]

Note. [a] Student's *t*-test for difference between the exposed and control groups. [b] Chi-square tests for differences in distributions between the exposed and control groups.

Airborne PAH Monitoring

As shown in Table 2, the median value of the eight carcinogenic PAHs in the air of the working places was 0.852 µg/m³ (range 0.259-3.330 µg/m³) for the exposed group and 0.306 µg/m³ (range 0.233-0.350 µg/m³) for the control group, and the difference was statistically significant (*P*=0.008). The airborne median level of B[*a*]P alone was 0.100 µg/m³ (range 0.028-0.360 µg/m³) for the exposed group and 0.040 µg/m³ (range 0.020-0.060 µg/m³) for the control group, and the difference was also statistically significant (*P*=0.014).

Levels of BPDE-albumin Adducts

As shown in Table 2, the BPDE-albumin adduct level in coke oven workers was significantly higher (42.10 fmol/mg albumin, median) than that in controls (14.16 fmol/mg albumin, median, *P*=0.000). The effects of occupational exposure to PAHs on BPDE-albumin adducts which were assessed using a logistic regression model with adjustment for possible confounders (i.e., age, smoking status, and alcohol use) are shown in Table 3. The OR of the exposure group having higher BPDE-albumin adducts was 1.79 (95% CI=1.10-2.92, *P*=0.019). No significant modification effects of any confounders in the logistic regression model were found during the assessment by the analysis of interactions.

Urinary Levels of 1-OHP

The levels of 1-OHP in the urine of the coke oven workers were higher than those in the urine of controls (*P*=0.001, Table 2). The median of 1-OHP in coke oven workers and controls was 5.46 and 2.96 µmol/mol creatinine, respectively. The OR for PAH exposure associated with 1-OHP levels was also assessed using a logistic regression model with adjustment for possible confounders (i.e., age, smoking status, and alcohol use). The PAH exposure was associated with a significantly increased risk of having higher 1-OHP levels (OR=2.45; *P*=0.049, Table 3).

TABLE 2

BPDE-albumin Adduct and Urinary 1-OHP Levels Between the Exposed and Control Groups

Variables	Exposed Group	Control Group	P Value[a]
Eight Carcinogenic PAHs (Median Levels, Range)	0.852 (0.259-3.330)	0.306 (0.233-0.350)	0.008
B[*a*]P (Median Levels, Range)	0.100 (0.028-0.360)	0.040 (0.020-0.060)	0.014
BPDE-Albumin Adducts Levels (Median, 25th-75th)	42.10 (19.86-65.86)	14.16 (1.79-36.20)	0.000
1-OHP Levels (Median, 25th-75th)	5.46 (2.87-14.62)	2.96 (0.69-6.03)	0.001

Note. [a] Mann-Whitney test for differences between the exposed and control groups.

TABLE 3

Multivariate Logistic Regression Analysis of BPDE-albumin Adduct and 1-OHP Levels With Adjustment for Confounding Factors

Variables Included in the Model	BPDE-albumin Adduct Levels[a]		1-OHP Levels[b]	
	OR (95% CI)[g]	P Value	OR (95% CI)[g]	P Value
Exposure to PAHs[c]	1.79 (1.10-2.92)	0.019	2.45 (1.01-5.96)	0.049
Age[d]	1.39 (0.85-2.28)	0.194	1.18 (0.46-2.99)	0.735
Smoking Status[e]	1.16 (0.65-2.06)	0.610	0.90 (0.27-3.04)	0.863
Alcohol Users[f]	0.91 (0.68-1.44)	0.676	1.19 (0.49-2.88)	0.703

Note. [a] BPDE-albumin adduct levels, >28.64 (1), ≤28.64 (0); [b] 1-OHP levels, >1.01 (1), ≤1.01 (0); [c] PAH exposed group (1), control group (0); [d] age, >39.0 (1); ≤39.0 (0); [e] Smoking status, >Yes (1), No (0); [f] Alcohol users, Yes (1), No (0); [g] OR: odds ratio; CI: confidence interval.

Correlations Between BPDE-albumin Adduct and Urinary 1-OHP Levels

The study has demonstrated a relationship between the BPDE-albumin adduct and urinary 1-OHP levels. There was a significantly positive correlation between the BPDE-albumin adduct and urinary 1-OHP levels in all subjects (Spearman's correlation, r_s=0.395; *P*=0.000).

DISCUSSIONS

In this study, we investigated whether BPDE-albumin adduct is a useful long-term biomarker of PAH exposure in coke oven workers. Our results showed that exposure to PAHs resulted in a significant increase in the levels of BPDE-albumin adducts in the exposed group compared with those in the control group, and this increase was not affected by age, smoking status, and alcohol consumption. Relatively few studies have used blood protein adducts as biomarkers of exposure to PAHs. The finding of an increase in BPDE-albumin adducts in workers due to exposure to PAHs in the present study is consistent with other investigations[6-7], although Omland *et al.*[14] and Kure *et al.*[15] have shown no statistical significance in the levels of B[*a*]P-albumin adducts between exposed and control groups and no contribution of occupational exposure to PAHs to the formation of adducts. The lack of association between BPDE-albumin adducts and smoking is consistent with a previous study[16]. This is perhaps because the level of B[*a*]P in cigarette smoke is much lower than that in coke oven emissions.

The urinary 1-OHP is one of the most commonly used biomarkers in the assessment of occupational exposure to PAHs. High exposure to pyrene in workplaces has been associated with increased levels of 1-OHP in the urine. Pyrene is a common PAH and the largest proportion of pyrene is present in the volatile phase. In the present study, the 1-OHP levels in coke oven workers were significantly higher than those in controls. Smoking status did not influence the differences between the exposed and control groups, probably because the exposure to PAHs in coke oven workers is overwhelmingly higher compared with exposure to smoking. Zhang *et al*. have obtained similar results[17].

In this study, a significant correlation was observed between the levels of BPDE-albumin adducts and urinary 1-OHP. The possible reasons for the relatively weak correlation between these two biomarkers are as follows. 1-OHP is based on a single spot-urine sample reflecting only the recent exposure and represents the exposure within the last 24 hours, whereas BPDE-albumin adduct is an internal dose marker of cumulative exposure and has a residence time of 1 month in blood. Pyrene is almost exclusively metabolized to 1-OHP. On the contrary, BPDE-albumin adducts may be chemical-specific and caused by the metabolic activation of B[*a*]P. Pyrene resulting in the formation of 1-OHP is not detectable through BPDE-albumin adducts.

In conclusion, these data reinforce the notion that plasma BPDE-albumin adducts and urinary 1-OHP are useful biomarkers of exposure to PAHs. Formation of BPDE-albumin adducts is associated with urinary 1-OHP. BPDE-albumin adducts reflect PAH exposure and metabolic activation over a 1-month life span of albumin, therefore, BPDE-albumin adduct is a useful biomarker for monitoring long-term exposure to PAHs.

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