Short Communication

Relationship of Exposure to Coke-Oven Emissions and Urinary Metabolites of Benzo(a)pyrene and Pyrene in Coke-Oven Workers

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Abstract

Coke-oven workers are occupationally exposed to a high concentration of polycyclic aromatic hydrocarbons (PAH), \( r^7 \)-7,9,10-Tetrahydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene (trans-anti-BaP-tetraol) and 1-hydroxy-pyrene (1-OHP) are urinary metabolites of benzo(a)pyrene and pyrene, respectively. In this study, we investigated the relationship among individual air exposure to benzene soluble fraction (BSF) of total particulates, as a surrogate marker of ambient PAH exposures, and urinary trans-anti-BaP-tetraol and 1-OHP concentrations in coke-oven workers at a steel plant in Taiwan. Fifty-seven subjects, including 41 male workers who work in one coke-oven plant and 16 men (referents) from an administrative area, were studied. The mean trans-anti-BaP-tetraol and 1-OHP concentrations (mean ± SD) were 0.4 ± 0.3 nmol/mol creatinine and 9.7 ± 21.6 μmol/mol creatinine, respectively, in coke-oven workers. These levels were significantly higher than those in referents (0.03 ± 0.03 nmol/mole creatinine, \( P < 0.001 \) and 0.4 ± 0.2 μmol/mol creatinine, \( P < 0.01 \), respectively).

Urinary trans-anti-BaP-tetraol concentrations were significantly and positively correlated with individual average BSF and urinary 1-OHP concentrations. That is, the higher the urinary trans-anti-BaP-tetraol concentrations, the more ambient BSF exposure and urinary 1-OHP concentrations (Spearman correlation coefficients \( r = 0.68 \) and 0.70, respectively; \( P < 0.0001; n = 57 \)). These findings suggest that urinary 1-OHP and trans-anti-BaP-tetraol might be considered as potential biomarkers for the assessment of uptake of known PAH carcinogens in the air.

Introduction

COEs containing PAHs, are formed and released into the environment when coal is pyrolysed into coke (1, 2). Thus coke-oven workers have a high probability of exposure to PAHs during the coke-making process. Some PAHs such as benzo(a)pyrene with four or more benzene rings are likely to be human carcinogens (1–3). In epidemiologic studies, workers with long-term exposures to COE were reported to have a significantly higher incidence of cancer, especially those of the lung and colon (4, 5).

Benzo(a)pyrene is commonly detected in virtually all of the PAH-containing mixtures. In the vicinity of coke-oven areas, these mixtures are composed of >100 different chemical compounds (1, 2). It has been demonstrated to be one of the most potent carcinogens among PAH. Because of its ubiquitous occurrence and strong carcinogenicity, it is regarded frequently as a surrogate marker for other PAHs. Benzo(a)pyrene is metabolized by cytochromes P450 to form arene oxides and phenols (6, 7).

The arene oxides may rearrange spontaneously to phenols or undergo hydration catalyzed by epoxide hydrolase, leading to dihydrodiols. 7,8-Dihydro-7,8-dihydroxybenzo(a)pyrene is additionally oxidized to anti- and syn BPDE by P450s and other enzymes. Among the four BPDE enantiomers produced in these reactions, the \( (7R,8S,9S,10R) \)-enantiomer of anti-BPDE is formed to the greatest extent and shows the highest carcinogenic activity. Our preliminary study showed that trans-anti-BaP-tetraol but not \( r^7 \)-7,9,10-tetrahydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene or \( r^7 \)-7,8,9,10-tetrahydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene could be detected in the urine of coke-oven workers (7).

The four-ring PAH pyrene has been reported to be present at even higher concentrations than the five-ring PAH benzo(a)pyrene and is present in both the gas and particle phase on the top of coke-ovens (1). In humans, pyrene is almost exclusively metabolized to 1-OHP (3). Our earlier study found that urinary 1-OHP concentrations were significantly correlated with the ambient BSF of total particulates (Spearman correlation coefficient \( r = 0.47 \); \( P < 0.001 \)) in 80 coke-oven workers. A 10-fold increase in average air BSF resulted in a ∼2.5-fold increase in 1-OHP concentrations (8).

As part of a program to investigate human metabolism of benzo(a)pyrene, we developed a sensitive and specific method to quantify trans-anti-BaP-tetraol in urine using gas chromatography/negative ion chemical ionization/mass spectrometry (7). In the present study, we used this method to examine the relationship of individual urinary trans-anti-BaP-tetraol con-

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3 The abbreviations used are: COE, coke-oven emission; BPDE, 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene; BSF, benzene soluble fraction of total particulates; 1-OHP, 1-hydroxy-pyrene; PAH, polycyclic aromatic hydrocarbon; trans-anti-BaP-tetraol, \( r^7 \)-7,9,10-tetrahydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene.
centration to ambient BSF exposure and 1-OHP concentrations in the urine of coke-oven workers at a steel plant in Taiwan.

Materials and Methods

Study Subjects. Study subjects were men employed in a large steel company in southern Taiwan. The detailed study design has been described previously (9). All of the selected coke-oven workers were currently working and had been employed at least 3 months in the coke-oven plant. The referents were officers who had not visited either coke operation area in the past 3 months and worked only in a large administrative area in the same company, ~2 km away from the coke operation areas. The study population included 41 coke-oven workers and 16 referents who had provided enough urine for detecting both trans-anti-BaP-tetraol and 1-OHP concentrations. The study was approved by the Institutional Review Board of the Harvard School of Public Health.

Ambient BSF. Individual personal breathing-zone air samples for BSF determinations were collected by battery-operated personal air sampling pumps (SKC, model 224PCXR7) for 3 consecutive workdays between August 1995 and February 1996 (9). A two-piece cassette was attached to the lapel of the workers, and air was drawn through it. The cassette contained 37-mm preweighed glass fiber filters (pore size 0.8 μm; Gelman Sciences). Each sampling apparatus was checked at least twice a day and the flowmeter reading recorded. Most subjects were sampled for at least 6 h during the complete workday. Airflow calibrations were maintained within ±5% of 2 liters/min. The mean total air volume was ~880 liter/sampling (10).

All of the samples were analyzed for BSF using United States Occupational Safety and Health Administration analytical methods (11). Exposure was also categorized by work area: topside oven workers, sideoven workers, and referents (12). The limit of detection and reliable quantitation limit of the overall procedure were 2.1 and 10.9 μg, respectively. Levels below the detection limit (4% and 38% of measurements in the exposed group and referents, respectively) were treated as 1 μg, half the detection limit (9). One benzene-soluble pooled sample from the topside oven workers was analyzed to determine the PAH composition using a Hitachi (Tokyo, Japan) high-performance liquid chromatograph equipped with a 250 mm × 4 mm inner diameter ChroCART 250-4 LiChropher PAH column (E. Merck, Armstadt, Germany; Ref. 10).

Analysis of Urine for 1-OHP and trans-anti-BaP-Tetraol. Spot urine samples were collected at the end of the work shift during the air sampling. Urine samples were analyzed for 1-OHP by high performance liquid chromatography with fluorescence detection, described in detail elsewhere (8). The limit of detection of urinary 1-OHP was 1.9 pmol/ml determined as the mean ± 3 SD of 10 urine samples from unexposed urban residents. The measurements below a concentration of 1.9 pmol/ml were set at 0.9 pmol/ml, half of the detection limit. The concentration of urinary 1-OHP is presented in units of μmol/mol creatinine.

The rest of the urine samples were shipped with dry ice by air overnight to Dr. Stephen Hecht’s laboratory for analysis of trans-anti-BaP-tetraol. These samples were stored at −30°C until analysis. The analyses were carried out by derivatization and gas chromatography/negative ion chemical ionization/mass spectrometry, as described previously (7). The samples were analyzed without knowledge of their origin. The urine samples were divided into five sets for analysis of trans-anti-BaP-tetraol. Each set consisted of the following: (a) two assay blanks (water); (b) three positive control samples; and (c) 10–12 urine samples from the study subjects. The positive-control urine samples were generated by adding a known amount of trans-anti-BaP-tetraol to a urine sample from an individual with no known exposure to elevated levels of benzo(a)pyrene.

Before analysis, urine samples were vortexed to ensure sample homogeneity. Approximately 10 ml of urine was poured into a tared 50-ml glass centrifuge tube, and the exact amount of sample transferred was determined gravimetrically. The analysis of samples for trans-anti-BaP-tetraol was carried out as described previously (7). r-7, r-8, r-9, c-10-tetrahydroxy-7,8,9,10-tetrahydrolo[1H2]-benzo(a)pyrene was added to the urine sample before analysis as internal standard.

The mean recovery of internal standard in the urine samples of the study subjects was 19 ± 7%. The instrumental limit of detection was calculated to be ~0.35 fmol on column (with signal/noise = 2) which translates to ~0.5 pmol/ml assuming a 10-ml urine sample and overall recovery of 20% for the assay. Trans-anti-BaP-tetraol was detected in 3 (19%), 33 (87%), and 3 (100%) of 16 referents, 38 sideoven workers, and 3 topside oven workers. The measurements below the detection limit in each set of urine samples were set at half of the detection limit.

Urinary creatinine was determined as the creatinine-picrate complex by spectrophotometry (Hitachi U-2000, Tokyo, Japan) using a wavelength of 520 nm (8).

Statistical Methods. Mean and standard deviation as well as median and range were used to describe the distribution of age, Quetelet’s index, air average BSF, and urinary trans-anti-BaP-tetraol and 1-OHP concentrations by the status of exposure. Smoking status (yes versus no), alcohol consumption (yes versus no), CYP1A1 MspI polymorphism (tertiles), and regular use of respirators (yes versus no) were also examined. Measurements of BSF (μg) below the detection limit were assigned as half of the detection limit before dividing by the total amount of air sampled (m3). The same principle was also applied for urinary trans-anti-BaP-tetraol (fmol/ml) and 1-OHP (pmol/ml), with concentrations below the limit of detection being set at half the detection limit before dividing by urinary creatinine concentration (mmol/ml).

Student’s t statistics were used to compare BSF exposures and urinary trans-anti-BaP-tetraol and 1-OHP concentrations between coke-oven workers and referents. Spearman correlation coefficients were used to study the relationship of urinary trans-anti-BaP-tetraol concentrations to air average BSF and urinary 1-OHP concentrations.

Air average BSF, urinary 1-OHP, and trans-anti-BaP-tetraol concentrations were log10-transformed to normalize their distributions before regression analysis. The relationship of urinary trans-anti-BaP-tetraol concentrations to air average BSF or urinary 1-OHP concentrations was investigated by multiple linear regression models after adjusting for other variables. The data were analyzed using the SAS statistical package (13). All of the Ps are two-sided.

Results

PAH in BSF from the topside oven workers included acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, and dibenzo(a,h)anthracene (10). Demographic data and concentrations of ambient BSF, urinary trans-anti-BaP-tetraol, and 1-OHP categorized by exposure groups are shown in Table 1.
There were no significant differences in age, Quetelet’s index, smoking status, and alcohol consumption between coke-oven workers and referents. Among the coke-oven workers, ambient BSF concentration (mean \( \pm \) SD) was 105.1 \( \pm \) 147.1 \( \mu \)g/m\(^3\), significantly higher than in referents (11.2 \( \pm \) 10.7 \( \mu \)g/m\(^3\); \( P < 0.001 \)). Trans-anti-BaP-tetraol was detected in 36 of 41 (87.8%) coke-oven workers and 3 of 16 (18.8%) referents.

Urinary trans-anti-BaP-tetraol concentrations were highly correlated with individual average BSF and urinary 1-OHP concentrations. The more the urine trans-anti-BaP-tetraol concentrations, the higher were the ambient BSF exposure and urinary 1-OHP concentrations (Spearman correlation coefficients \( r = 0.68 \) and 0.70, respectively; \( P < 0.0001 \); \( n = 57 \); Fig. 1). These results remained similar when we excluded subjects \( n = 18 \) in whom the urinary trans-anti-BaP-tetraol concentrations were below the detection limit (Spearman correlation coefficients \( r = 0.53, P = 0.0005 \) and \( r = 0.57, P = 0.0002 \), respectively; \( n = 39 \)). Among the 36 coke-oven workers with detectable urinary trans-anti-BaP-tetraol, urinary trans-anti-BaP-tetraol concentrations were also significantly correlated with individual average BSF and urinary 1-OHP concentrations (Spearman correlation coefficients \( r = 0.43, P = 0.006 \) and \( r = 0.34, P = 0.03 \), respectively). Urinary 1-OHP levels were \( \sim 10,000 \)-fold higher than urinary trans-anti-BaP-tetraol levels in the same subjects (Fig. 1).

In multiple linear regression with log\(_{10}\)-transformed urinary trans-anti-BaP-tetraol concentrations and ambient BSF exposure, these findings were not influenced by other variables, including age, Quetelet’s index, smoking status, and alcohol consumption. A 10-fold increase in air average BSF resulted in a \( \sim 6 \)-fold increase in urinary trans-anti-BaP-tetraol concentrations \( [10^{\log_{10}10} = 6; P < 0.0001] \).

Since most referents had no detectable trans-anti-BaP-tetraol levels in urine (13 of 16 subjects), we examined the effect of smoking and CYP1A1 MspI polymorphisms in coke-oven workers \( n = 41 \). There was no significant difference between urinary trans-anti-BaP-tetraol concentrations in smokers and nonsmokers (mean \( \pm \) SD: 0.30 \( \pm \) 0.21 in 21 smokers versus 0.40 \( \pm \) 0.29 in 20 nonsmokers; \( P = 0.21 \)). Coke-oven subjects with homozygous variant CYP1A1 MspI had a slightly higher trans-anti-BaP-tetraol concentrations in urine (mean \( \pm \) SD: 0.42 \( \pm \) 0.28; \( n = 5 \)) than those of heterozygous variant type (0.34 \( \pm \) 0.29; \( n = 23 \)) and wild type (0.33 \( \pm \) 0.19; \( n = 13 \)), but these values were not significantly different (\( P = 0.80 \)).

**Discussion**

We found a statistically significant correlation among individual ambient BSF exposures, urinary 1-OHP, and urinary trans-anti-BaP-tetraol. Although we identified both pyrene and benzo(a)pyrene in BSF of the topside oven workers (10), we did not have information about individual PAH exposure. Benzo(a)pyrene is one of the more potent carcinogens in PAH mixtures, and levels of trans-anti-BaP-tetraol in urine provide an index of benzo(a)pyrene uptake (7). Pyrene occurs in higher concentrations in PAH mixtures than benzo(a)pyrene but is noncarcinogenic (3). Pyrene may be distributed between the gas and particulate phase, whereas benzo(a)pyrene is probably found only in the particulate phase of COE. The correlation between trans-anti-BaP-tetraol and 1-OHP indicates that the latter is a good biomarker of carcinogen uptake and a valuable dosimeter of human internal exposure to carcinogenic PAH.

Among the coke-oven workers examined, we were unable to find a significant difference in urinary trans-anti-BaP-tetraol levels in smokers and nonsmokers. Our earlier study reported that urinary trans-anti-BaP-tetraol levels were considerably lower in subjects exposed to cigarette smoke than in psoriasis patients and coke-oven workers (7). Benzo(a)pyrene concentrations in mainstream cigarette smoke are \( \sim 9 \) ng/cigarette (14). We found previously that urinary trans-anti-BaP-tetraol was detectable in only 9 of 21 smokers (43\%) who smoked an average of 27 cigarettes/day (7). Mean trans-anti-BaP-tetraol in

### Table 1: Demographic data by exposure situations among coke-oven workers and referents

<table>
<thead>
<tr>
<th></th>
<th>Coke-oven workers ( n = 41 )</th>
<th>Referents ( n = 16 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>42.1 ± 9.5 (18, 42, 59)</td>
<td>41.8 ± 4.3 (34, 42, 47)</td>
</tr>
<tr>
<td>Quetelet’s index (kg/m(^2))</td>
<td>24.1 ± 3.3 (17.8, 23.9, 33.7)</td>
<td>22.8 ± 2.2 (19.9, 22.4, 26.7)</td>
</tr>
<tr>
<td>BSF (( \mu )g/m(^3))</td>
<td>105.1 ± 147.1 (10.5, 59.2, 916.4)</td>
<td>11.2 ± 10.7 (1.0, 7.2, 37.4)</td>
</tr>
<tr>
<td>trans-anti-BaP-tetraol (nmol/mol creatinine)</td>
<td>0.4 ± 0.3 (0.005, 0.3, 1.3)</td>
<td>0.03 ± 0.03 (0.005, 0.015, 0.1)</td>
</tr>
<tr>
<td>1-OHP (nmol/mol creatinine)</td>
<td>9.7 ± 21.6 (1.2, 5.5, 142.6)</td>
<td>0.4 ± 0.2 (0.06, 0.4, 0.8)</td>
</tr>
<tr>
<td>Smoking status (^a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>20 (48.8)</td>
<td>10 (62.5)</td>
</tr>
<tr>
<td>Yes</td>
<td>21 (51.2)</td>
<td>6 (37.5)</td>
</tr>
<tr>
<td>Alcohol consumption (^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>32 (78.0)</td>
<td>12 (75)</td>
</tr>
<tr>
<td>Yes</td>
<td>9 (22.0)</td>
<td>4 (25)</td>
</tr>
<tr>
<td>CYP1A1 MspI (^c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>13 (31.7)</td>
<td>7 (43.8)</td>
</tr>
<tr>
<td>Heterozygous variant</td>
<td>23 (56.1)</td>
<td>6 (37.5)</td>
</tr>
<tr>
<td>Homozygous variant</td>
<td>5 (12.2)</td>
<td>3 (18.8)</td>
</tr>
<tr>
<td>Regular use of respirators (^c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>12 (29.3)</td>
<td>–</td>
</tr>
<tr>
<td>Yes</td>
<td>29 (70.1)</td>
<td>–</td>
</tr>
</tbody>
</table>

\(^a\) Mean ± SD (Min., med., max).

\(^b\) Information was reported in the week prior to urine specimen collection.

\(^c\) No. (%)
that study was 0.5 fmol/ml, well below the levels found in coke-oven workers in the present study, which accounts for our inability to detect a difference between coke-oven workers who did or did not smoke. In the present study, the referents smoked a maximum of 15 cigarettes/day, which explains why \( \text{trans-anti-BaP-tetraol} \) was detected only in 3 of 16 referents (19%). In addition, we did not find any significant effect of \( \text{CYP1A1/MspI polymorphism} \) on urinary \( \text{trans-anti-BaP-tetraol} \) levels perhaps because of small sample size used in this study.

In summary, we found correlations among ambient BSF exposure, urinary \( \text{trans-anti-BaP-tetraol} \), and 1-OHP concentrations. PAHs are present in complex mixtures of >100 different compounds in the vicinity of coke-oven areas (1). It is not feasible to evaluate each constituent independently in the atmosphere or by biological monitoring in epidemiologic studies. Our findings indicated that urinary 1-OHP and \( \text{trans-anti-BaP-tetraol} \) could be used as suitable biomarkers to assess known PAH carcinogens in air. Urinary 1-OHP is more readily determined and may be regarded as a surrogate for \( \text{trans-anti-BaP-tetraol} \). Moreover, since the latter is a product of the major metabolic activation pathway of BaP, urinary 1-OHP might be regarded as a biomarker of risk. Additional studies are required to investigate this hypothesis. It would also be important to generalize our findings to populations with lower exposure to PAH.

**References**

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