Repeate Measurements of Urinary Methylated/DNA Lesions, Acute Toxicity, and Mutagenicity in Coke Oven Workers

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Abstract

We conducted a repeated-measures cohort study of coke oven workers to evaluate the relationships between the traditional exposure biomarker, urinary 1-hydroxypyrene (1-OHP), and a series of biomarkers, including urinary 8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxodG), N7-methylguanine (N7-MeG), acute toxicity, and mutagenicity. A total of eight spot urine samples were collected from each high-exposed (at topside oven area) and low-exposed workers (at side oven area) during the whole working cycle, which consisted of 6 consecutive days of working followed by 2 days off. Our results showed that the high-exposed workers had significantly higher urinary levels of 1-OHP, 8-oxodG, and N7-MeG compared with the low-exposed workers. Acute toxicity and mutagenicity of urine were also found to be markedly increased in the high-exposed workers, as determined by Microtox assay and Ames test, respectively. Multivariate regressions analysis revealed that the urinary 8-oxodG, N7-MeG, or acute toxicity was significantly correlated with 1-OHP concentrations. Overall, the present study showed that exposure to coke oven emissions increased oxidatively damaged DNA products and mutagenicity of urine, and for the very first time, such exposure was also found to increase DNA methylation and urinary acute toxicity. The potential source of methylating agents in coke oven emissions warrants further investigation. Additionally, with repeated measurements, the pattern of time course for urinary 1-OHP was found to be different from those of 8-oxodG and N7-MeG, as well as acute toxicity and mutagenicity. This finding implies that the single measurement that was often conducted in occupational healthy investigations should be used with certain precautions, because single measurement may fail to provide the proper information of interest. (Cancer Epidemiol Biomarkers Prev 2008;17(12):3381–9)

Introduction

Coke oven emissions are human carcinogens (1). Epidemiologic studies have shown that the workers with long-term exposure to coke oven emissions have a high incidence of cancer, especially lung, colon, prostate, and bladder cancers (2, 3). Coke oven emissions are complex chemical mixtures that include polycyclic aromatic hydrocarbons, nitrosamines, coal tar, arsenic compounds, and benzene (4). Therefore, identification of early biomarkers for occupational exposure to coke oven emissions may lead to effective preventive measures to reduce exposure and the related health effects (5).

It has been shown that the carcinogens (e.g., polycyclic aromatic hydrocarbons and arsenic) exert their biological effects probably through the generation of reactive oxygen species. These excess reactive oxygen species can lead to the formation of oxidative damage to DNA. Among the most abundant oxidatively damaged DNA is 8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxodG), which was found to induce mutation through G to T transversion (6). In the past decade, several studies using 8-oxodG as a biomarker of oxidative injury have found significantly higher 8-oxodG levels in leukocyte DNA or urine of workers exposed to coke oven emissions compared with those of nonexposed workers (5, 7). We have also shown previously a significant correlation between urinary 1-hydroxypyrene (1-OHP, a major metabolite of pyrene) and 8-oxodG for coke oven workers (8).

Among these known carcinogens present in coke oven emissions, nitrosamines have not received much attention thus far although the carcinogenic effect of nitrosamines has been well shown (9). Bioactivation of nitrosamines can produce reactive intermediates, which can react with nucleophilic nitrogen and oxygen atoms in DNA (10). A number of DNA adducts could be formed, including those alkylated at the O6, N2, N-7, and N-3 positions of guanine; the N-7 and N-3 positions of adenine; the O2 position of cytosine; and the O3' position of thymine as well as the phosphodiester backbone (11, 12). As the N7 position of guanine is the predominant reaction site, N7-methylguanine (N7-MeG) has been suggested to be a useful biomarker of exposure.
to endogenous and exogenous methylating agents such as nitrosamines (13). Although N7-MeG adducts are not considered to be promutagenic, they are hydrolytically unstable, undergoing spontaneous depurination to produce apurinic sites and single-strand breaks in DNA, and are excreted in urine. If not repaired, apurinic sites can potentially cause G to T transversions (14). Previously, urinary excretion of N7-MeG has been found to be increased in laboratory animals after exposure to methylating agents (15), and the N7-MeG in urine has been shown to be higher among smokers than among non-smokers, probably resulting from exposure to tobacco-specific nitrosamines (16, 17). Although nitrosamines do exist in coke oven emissions, no information was available on the urinary N7-MeG levels of workers related to the occupational exposure to coke oven emissions.

Chemical analysis alone may not provide sufficient information to assess the potential effect of various hazard exposures. Thus, biological monitoring in the form of a battery of toxicity and mutagenicity tests is becoming an essential supplement to chemical analysis in urine (18). Measurement of urinary mutagenicity by Ames test has been widely used to detect absorption of potentially mutagenic compounds as a nonspecific biomarker of exposure. Increased urinary mutagenicity has been reported in smokers and workers exposed to aromatic amines and benzidine in the rubber and dyes industries (19-21). An elevated urinary mutagenicity was also observed among groups occupationally exposed to polycyclic aromatic hydrocarbons, including coke oven workers, chimney sweeps, and aluminum workers (22), although the negative findings on mutagenic activity in urine samples of workers exposed to polycyclic aromatic hydrocarbons have also been reported by several authors (23, 24). Meanwhile, because many chemicals that are mutagenic (or carcinogenic) are also toxic, acute toxicity of urine has been used as a screening test in addition to mutagenicity test (18). One of the urinary acute toxicity tests is the assay system that uses the bioluminescent bacteria as a test organism. When harmful substances are present, their bioluminescent decreases, and the degree of toxicity is proportional to the light loss. Due to its ease of operation, low cost, standardization, reproducibility, and sensitivity, this bioassay could be applied to assess the occupational exposure to toxic chemicals (18). It is noted, however, that until now no information was available on the urinary acute toxicity of coke oven workers.

The aim of this study was to investigate associations between coke oven emission exposure and urinary methylated/oxidative DNA lesions, acute toxicity, and mutagenicity in coke oven workers. We conducted a repeated-measures cohort study in a coke oven plant. Because coke oven emissions comprise mainly polycyclic aromatic hydrocarbons (25), the well-validated polycyclic aromatic hydrocarbon exposure biomarker 1-OHP was measured to serve as an indirect indicator of exposure to coke oven emissions. Possible sources of methylating agents present in coke oven emissions were also discussed.

Materials and Methods

Study Design and Subjects. This study was approved by the Institutional Review Board of Kaohsiung Medical University. This study was a repeated-measures short-term prospective study of coke oven workers consisting of 37 male workers, including 15 topside oven workers (high-exposed group) and 22 side oven workers (low-exposed group). A working cycle consisted of six consecutive working days followed by two days off. Topside oven workers were defined as staying at topside >7 hours per day, and their office room was located on the topside. Side oven workers were defined as staying at the side oven >4 hours per day, and the office was located around the side oven. All workers have been used ≥1 y in the same coke oven plant.

Data Collection. A standardized questionnaire was used to collect demographic information about occupational and medical history; smoking and alcohol drinking habits; and potential confounding factors. Eight spot urine samples were collected from each subject during the whole working cycle as follows (Fig. 1): sample 1, preshift on day 1 (D1-Pre); sample 2, postshift on day 1 (D1-Post); sample 3, postshift on day 3 (D3-Post); sample 4, preshift on day 6 (D6-Pre); sample 5, postshift on day 6 (D6-Post); sample 6, morning on off-day 1 (D7-Morn); sample 7, morning on off-day 2 (D8-Morn); and sample 8, preshift on day 1 of the next working cycle (D9-Pre). All urine samples were frozen at −20°C until urinary 1-OHP, 8-oxodG, N7-MeG, acute toxicity, and mutagenicity analyses were done. Urinary creatinine was measured for each sample using spectrophotometry (U-2000; Hitachi) at a wavelength of 520 nm (25).

Urinary 1-OHP Analysis. Urinary 1-OHP was determined by a high performance liquid chromatography (HPLC) with the fluorescence detection method (26). Briefly, 20 mL of 0.1 mol/L acetate buffer (pH 5.0) and 20 µL glucuronidase-arylsulfatase (134,600 unit/mL; Sigma) were added to 10 mL urine and incubated for 4 h at 37°C in an electronically controlled rotary shaking bath. The mixture was then applied to a Sep-Pak C18 cartridge (500 mg/3 mL; Waters) preconditioned with 3 mL methanol and 6 mL of distilled water. The column was then washed with 5 mL of 0.1 N sodium acetate. The fraction containing 1-OHP was eluted with 2 mL of 2-propanol, collected, dried under vacuum for 2 h, and dissolved in 1 mL of 2-propanol. Twenty microliters of the sample solution were injected into the HPLC system. The HPLC system consisted of an autoinjector (Hewlett-Packard 1100), a Hitachi model L-7100 pump, and a Hitachi model L-7485 fluorescence detector with excitation wavelength 241 nm and emission wavelength 388 nm. A C18 analytic column (250 × 4.6 mm; Supelco) was used. The gradient mode was used to achieve the separation of analytes using mixtures of mobile phase A (75% methanol) and mobile phase B (100% methanol) at a flow rate of 1 mL/min. The following gradient was run: 0 to 15 min, 0% mobile phase B; 15 to 16 min, 100% mobile phase B; 16 to 17 min, 100% mobile phase B; 17 to 18 min, 0% mobile phase B; and 18 to 23 min, 0% mobile phase B. The retention time for 1-OHP was 16.2 min. The limit of detection of urinary 1-OHP was ~0.16 ng/mL. The concentration of individual urinary 1-OHP was adjusted to the urinary concentration of creatinine (ng/mg creatinine) to control for variation in urinary output.

Urinary 8-oxodG Analysis. Urinary 8-oxodG concentrations were measured using a validated method of liquid chromatography/tandem mass spectrometry.
(LC-MS/MS) with on-line solid-phase extraction as recently reported by Hu et al. (27). Briefly, 20 μL of urine was diluted 10-fold with 5% methanol containing 0.1% formic acid. After the addition of 40 μL of $^{15}$N$_7$-8-oxodG solution (20 μg/L in 5% methanol/0.1% formic acid) as internal standard, a 100 μL of prepared urine sample was directly injected into the on-line solid-phase extraction LC-MS/MS. After automatic sample cleanup, LC-MS/MS analysis was done using a PE Series 200 HPLC system interfaced with a PE Sciex API 3000 triple quadrupole mass spectrometer with electrospray ion source. The samples were analyzed in the positive ion multiple reaction monitoring mode, and the transitions of the precursors to the product ions were as follows: m/z 284→168 (quantifier ion) and 284→140 (qualifier ion) for 8-oxodG, and m/z 289→173 (quantifier ion) and 289→145 (qualifier ion) for $^{15}$N$_7$-8-oxodG. With the use of isotopic internal standards and on-line solid-phase extraction, this method had a high sensitivity with limit of detection of 5.7 ng/L (2.0 fmol) on column. The concentration of individual urinary 8-oxodG was adjusted to the urinary concentration of creatinine (ng/mg creatinine) to control for variation in urinary output.

**Urinary N7-MeG Analysis.** Urinary N7-MeG concentrations were measured using a published method of LC-MS/MS with on-line solid-phase extraction (16). Fifty microliters of urine were diluted 1,000 times with 96% acetonitrile containing 0.1% formic acid. A 100-μL aliquot of diluted urine was added to 100 μL of $^{15}$N$_7$-N7-MeG solution (0.62 ng/mL) as internal standard, then mixed and injected into the same on-line solid-phase extraction LC-MS/MS system described above. The samples were analyzed in the positive ion multiple reaction monitoring mode, and the transitions of the precursors to the product ions were as follows: m/z 166→149 (quantifier ion) and 166→124 (qualifier ion) for N7-MeG, and m/z 171→153 for $^{15}$N$_7$-N7-MeG. With the use of isotopic internal standards and on-line solid-phase extraction, this method had a high sensitivity with limit of detection 8.0 ng/L (4.8 pmol) on-column. The concentration of individual urinary N7-MeG was adjusted to the urinary concentration of creatinine (ng/mg creatinine) to control for variation in urinary output.

**Extraction of Urine Samples for Acute Toxicity and Mutagenicity Tests.** After thawing at room temperature, 12 mL of urine was loaded into a Sep-Pak C18 cartridge (500 mg/3 mL; Waters) preconditioned with 6 mL methanol and 6 mL distilled water. The cartridge was then washed with 6 mL of distilled water to withdraw the residual urine and histidine. The organics were eluted with 9 mL of methanol, evaporated to dryness with a SpeedVac system, and dissolved in 120 μL of DMSO to make a 100× concentrate. Urine extracts were kept at −20°C in the dark until use.

**Urinary Acute Toxicity Test Using Microtox Bioassay.** Microtox is a commercial toxicity bioassay based on the reduction in bioluminescence of luminescent bacteria (Vibrio fischeri). *V. fischeri* produces light as a byproduct of cellular respiration. When exposed to a toxicant, the rate of light production is reduced in proportion to the sample toxicity. A Microtox model 500 analyzer was used to run the tests for urine extracts in DMSO according to the “basic test” protocol using organic solvent sample solubilization (28, 29). The analyzer, reagents, and freeze-dried bacteria were obtained from Strategic Diagnostics, Inc. Phenol was used as the positive toxicity control for the Microtox assay. A mixture of 0.5% DMSO in Microtox diluent was prepared and used as the diluent for the test. An amount of 10 μL of urine extract (equivalent to 1 mL urine) was added to 1,990 μL of the Microtox diluent, making the initial concentration of sample of 0.5%. Reconstituted freeze-dried bacteria were exposed to a series of diluted urine extracts and incubated at 15°C. The luminescence inhibition after the 15-min exposure was taken as the end point. All Microtox data were recorded and analyzed by on-line software. Further necessary dilution was made according to the results of preliminary tests so that the data points in the Microtox plot were located on both sides of the effective concentration, EC$_{50}$, which is the concentration of urine extracts causing a 50% decrease in the light output. EC$_{50}$ values (expressed as mL equivalent of urine sample) were reported as the means of duplicate determinations. The test results were further expressed in toxicity unit (TU), which is 1/EC$_{50}$ and directly proportional to the toxicity. After adjustment for creatinine levels, the results were expressed as TU/mg creatinine.

**Urinary Mutagenicity Test.** The mutagenicity of urine concentrates was determined using the plate-incorporation preincubation technique on the *Salmonella typhimurium* TA98 strain in the presence of the microsomal fraction (59) of rat liver (50 μL per plate; ref. 30). The TA98 strain was chosen because this strain has been recommended in detecting the exposure to polycyclic aromatic hydrocarbons (31). Various doses of extract between 0.5 and 5 mL of urine equivalent were assayed. Revertant colonies were counted after a 48-h incubation at 37°C. DMSO was used as a solvent control to estimate the spontaneous frequency of revertants. Positive controls were benzo(a)pyrene (5 μg per plate) and 2-aminofluorene (5 μg per plate). Urinary mutagenic activity was taken as positive when at least one of the tested doses was able to double the number of revertants with respect to spontaneous revertants and expressed as the slope of the linear portion of the dose-response curve calculated by linear regression method, from at least two urine extract doses, as number of net revertants (rev)
per mL of urine (22). After adjustment for creatinine levels, the results were expressed as net rev/mg creatinine.

**Statistical Methods.** Mean and SD were used to describe the distributions of urinary 1-OHP, 8-oxodG, N7-MeG, acute toxicity, and mutagenicity as well as the demographic data for high- and low-exposed workers. The data were analyzed using the SAS statistical package (SAS version 9.1). Student's t and χ² statistics were used to compare urinary 1-OHP, 8-oxodG, N7-MeG, acute toxicity, and mutagenicity as well as other covariates between high- and low-exposed workers. Pearson’s correlation coefficients were used to study the relationship of urinary 8-oxodG, N7-MeG, acute toxicity, and mutagenicity to urinary 1-OHP concentrations. In multiple linear regression models, the relationship of urinary 8-oxodG, N7-MeG, acute toxicity, and mutagenicity to urinary 1-OHP concentrations were investigated after adjusting for potential confounding variables, such as age, body mass index (BMI), smoking status, and alcohol consumption.

**Results**

The demographic data for high-exposed workers and low-exposed workers are summarized in Table 1. High-exposed and low-exposed workers were similar in age (mean, 42.3 and 43.1 years, respectively) and BMI (mean, 23.9 and 24.2 kg/m², respectively). Of the high-exposed workers, 47% were current smokers compared with 55% in low-exposed workers. About 47% of the high-exposed workers reported being drinkers compared with 41% of the low-exposed workers. There were no significant differences in percentages of smokers and alcohol drinkers between the two groups.

The overall mean urinary concentrations of 1-OHP, 8-oxodG, and N7-MeG of the high-exposed and low-exposed workers during the entire working cycle are listed in Table 2. The overall mean urinary concentrations of 1-OHP, 8-oxodG, and N7-MeG were 5294, 3.09, and 3,803 ng/mg creatinine, respectively, for the high-exposed workers. As shown in Table 2, the overall mean urinary concentrations of 1-OHP, 8-oxodG, and N7-MeG during the entire working cycle were 55.0 versus 35.5 net rev/mg creatinine; P < 0.005). As for urinary mutagenicity, 61.7% and 46.6% of urine samples were clearly mutagenic for the high- and low-exposed workers, respectively. The high-exposed workers showed 1.5 times as much urinary mutagenicity than the low-exposed workers (55.0 versus 35.5 net rev/mg creatinine; P < 0.005).

The association among urinary 1-OHP, 8-oxodG, and N7-MeG as well as urinary acute toxicity and mutagenicity were analyzed using Pearson’s correlation coefficients. It was found that the urinary concentrations of 8-oxodG were highly associated with urinary 1-OHP concentrations (r = 0.36; P < 0.0001). A similar result was also found between the urinary N7-MeG and 1-OHP (r = 0.22; P = 0.0003). A slightly positive correlation between urinary acute toxicity and urinary 1-OHP concentrations was also observed, although it did not reach statistical significance (r = 0.13; P = 0.079). No correlation was found between urinary mutagenicity and 1-OHP in this study. Multivariate linear regression analyses were further done with adjustment for age, BMI, smoking, and alcohol drinking. As shown in Table 3, the correlation between urinary 8-oxodG (or N7-MeG) and urinary 1-OHP was not confounded by other variables (P < 0.0001 for 8-oxodG; P = 0.0008 for N7-MeG). Moreover, urinary acute toxicity was found to be slightly associated with 1-OHP (P = 0.042) and smoking (P = 0.019) while urinary mutagenicity was found to be associated with BMI (P = 0.018).

Figure 2 shows the time course of urinary concentrations of 1-OHP, 8-oxodG, and N7-MeG during the entire working cycle for both high-exposed and low-exposed workers. As shown in Fig. 2A, during the working days, the urinary concentrations of 1-OHP for the high-exposed workers increased dramatically with the number of working days and reached the top in day 6 (482.7 ng/mg creatinine, D6-Pre). On off-work days, the urinary 1-OHP levels decreased significantly and resulted in a reduction of 72% by the preshift of the next working cycle (135.1 ng/mg creatinine, D9-Pre). In contrast, among the low-exposed workers, the urinary 1-OHP levels remained around the mean value with little change (25.5-66.5 ng/mg creatinine) during the entire working cycle. As for the urinary 8-oxodG concentrations (Fig. 2B), the high-exposed workers at the start of the working cycle had relatively higher 8-oxodG levels (6.70-5.41 ng/mg creatinine, D1-D3) compared with the

**Table 1. Demographic characteristics of study subjects**

<table>
<thead>
<tr>
<th>Variable</th>
<th>High-exposed group: topside oven workers (n = 15)</th>
<th>Low-exposed group: sides oven workers (n = 22)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y*</td>
<td>42.3 ± 7.8</td>
<td>43.1 ± 10.5</td>
<td>0.410</td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>23.9 ± 2.8</td>
<td>24.2 ± 2.6</td>
<td>0.685</td>
</tr>
<tr>
<td>Cigarette smoking †</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>7 (47%)</td>
<td>12 (55%)</td>
<td>0.638</td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>8 (53%)</td>
<td>10 (45%)</td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption †</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinker</td>
<td>7 (47%)</td>
<td>9 (41%)</td>
<td>0.729</td>
</tr>
<tr>
<td>Nondrinker</td>
<td>8 (53%)</td>
<td>13 (59%)</td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± SD.
†Number (%).
Table 2. Overall mean urinary levels of multiple biomarkers in coke oven workers

<table>
<thead>
<tr>
<th>Variable</th>
<th>High-exposed group (mean ± SD)</th>
<th>Low-exposed group (mean ± SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-OHP (ng/mg creatinine)</td>
<td>296.2 ± 386.7</td>
<td>45.7 ± 45.9</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>8-oxodG (ng/mg creatinine)</td>
<td>4.83 ± 5.20</td>
<td>3.09 ± 2.50</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>N7-MeG (ng/mg creatinine)</td>
<td>5294 ± 4728</td>
<td>3803 ± 2246</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Acute toxicity (TU/mg creatinine)</td>
<td>12.6 ± 22.7</td>
<td>4.66 ± 4.05</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Mutagenicity (net rev/mg creatinine)</td>
<td>55.0 ± 49.0 (61.7%)*</td>
<td>35.5 ± 22.9 (46.6%)*</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>92.0 ± 40.2</td>
<td>97.9 ± 50.5</td>
<td>0.28</td>
</tr>
</tbody>
</table>

*Percentage of mutagenic samples: at least doubling the number of spontaneous revertants.

Discussion

In this study, we conducted a repeated-measures of 1-OHP, 8-oxodG, N7-MeG, acute toxicity, and mutagenicity in the urine of coke oven workers during the entire working cycle to evaluate the health burden from coke oven emission exposure. Notably, most previous studies measured urinary and/or blood biomarkers at the end of a work shift as a single measurement (5, 7, 26, 32, 33). These readings may be affected by the known circadian variation in certain biomarker level (e.g., 8-oxodG; ref. 34) and by other factors (e.g., age and diet; ref. 35). However, with the repeated sampling used in this study, we are able to minimize these effects due to the test subjects acting as their own controls (36) and offer a possibility to monitor the time course of DNA damage response and acute toxicity/mutagenicity in urine resulting from coke oven emission exposure.

Because coke oven emissions contain a large number of polycyclic aromatic hydrocarbons, numerous studies have focused on the correlation of external exposure to polycyclic aromatic hydrocarbons and its internal burden among workers exposed to coke oven emissions. Urinary 1-OHP, the major metabolite of pyrene, has been recommended as a sound biomarker to assess polycyclic aromatic hydrocarbon exposure (37). Previous studies have reported that urinary 1-OHP levels for coke oven workers worldwide ranged from 0.88 to 173 ng/mg creatinine at the end-of-shift concentration, except for Taiwan and China (reviewed in ref. 38). At the work place in Taiwan and China, coke oven workers have been reported to have the highest urinary 1-OHP concentration, up to 280 ng/mg creatinine. Our findings are consistent with the published results showing high

Table 3. Multivariate regression analysis for urinary 8-oxodG, N7-MeG, acute toxicity, and mutagenicity

<table>
<thead>
<tr>
<th>Variable</th>
<th>8-oxodG (b (SE))</th>
<th>8-oxodG (P)</th>
<th>N7-MeG (b (SE))</th>
<th>N7-MeG (P)</th>
<th>Urinary acute toxicity (b (SE))</th>
<th>Urinary acute toxicity (P)</th>
<th>Urinary mutagenicity (b (SE))</th>
<th>Urinary mutagenicity (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-OHP</td>
<td>0.005 (0.001)</td>
<td>&lt;0.0001</td>
<td>2.755 (0.809)</td>
<td>0.0008</td>
<td>0.011 (0.006)</td>
<td>0.042</td>
<td>0.023 (0.013)</td>
<td>0.087</td>
</tr>
<tr>
<td>Age</td>
<td>−0.026 (0.027)</td>
<td>0.298</td>
<td>33.83 (24.10)</td>
<td>0.162</td>
<td>−0.062 (0.148)</td>
<td>0.673</td>
<td>0.213 (0.404)</td>
<td>0.600</td>
</tr>
<tr>
<td>BMI</td>
<td>−0.089 (0.111)</td>
<td>0.423</td>
<td>88.31 (96.69)</td>
<td>0.361</td>
<td>1.046 (0.579)</td>
<td>0.073</td>
<td>3.960 (1.669)</td>
<td>0.018</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.497 (0.520)</td>
<td>0.340</td>
<td>538.8 (460.3)</td>
<td>0.243</td>
<td>6.613 (2.804)</td>
<td>0.019</td>
<td>−4.116 (6.859)</td>
<td>0.549</td>
</tr>
<tr>
<td>Alcohol drinking</td>
<td>0.412 (0.517)</td>
<td>0.427</td>
<td>491.6 (464.4)</td>
<td>0.291</td>
<td>2.202 (2.839)</td>
<td>0.439</td>
<td>−9.397 (6.848)</td>
<td>0.172</td>
</tr>
</tbody>
</table>

Abbreviation: b, regression coefficient.
Figure 2. Time course of urinary concentrations of 1-OHP (A), 8-oxodG (B), and N7-MeG (C) among workers exposed to coke oven emissions during the entire working cycle. Eight spot urine samples: D1-Pre, preshift on day 1; D1-Post, postshift on day 1; D3-Post, postshift on day 3; D6-Pre, preshift on day 6; D6-Post, postshift on day 6; D7-Morn, morning on off-day 1; D8-Morn, morning on off-day 2; and D9-Pre, preshift on day 1 of the next working cycle. Points, mean; bars, SE.
Points Morn, morning on off-day 2; and post shift on day 6; Post, 1; D1-Pre, preshift on day 1; D1-Post, postshift on day 1; D3-Post, postshift on day 3; D6-Pre, preshift on day 6; D6-Post, postshift on day 6; D7-Morn, morning on off-day 1; D8-Morn, morning on off-day 2; and D9-Pre, preshift on day 1 of the next working cycle. Points, mean; bars, SE.

Figure 3. Time course of urinary acute toxicity (A) and mutagenicity (B) among workers exposed to coke oven emissions during the entire working cycle. Eight spot urine samples: D1-Pre, preshift on day 1; D1-Post, postshift on day 1; D3-Post, postshift on day 3; D6-Pre, preshift on day 6; D6-Post, postshift on day 6; D7-Morn, morning on off-day 1; D8-Morn, morning on off-day 2; and D9-Pre, preshift on day 1 of the next working cycle. Points, mean; bars, SE.

urinary 1-OHP levels observed in Taiwan (at the end-of-shift, 402 and 59.6 ng/mg creatinine for topside and side oven workers, respectively).

It is interesting that the overall mean urinary 1-OHP level of topside oven workers was about 6 times higher than that of side oven workers (Table 2). This could be because topside oven workers had been exposed to ~7 times higher levels of airborne polycyclic aromatic hydrocarbons than side oven workers (~7,000 ng/m³ against ~950 ng/m³ using personal air-sampling device; ref. 39). Moreover, the personal hygiene behavior could have also resulted in a higher polycyclic aromatic hydrocarbon exposure in topside oven workers, because the time spent in wearing a respirator mask could be reduced especially when working under high-temperature conditions (40).

It is well established that the metabolic transformation of polycyclic aromatic hydrocarbons generates reactive electrophilic metabolites, causing DNA damage and inducing reactive oxygen species production (41, 42). In our study, the results showed that the high-exposed workers had significantly higher levels of urinary 8-oxodG than low-exposed workers. The urinary 8-oxodG levels were also highly correlated with urinary 1-OHP and not confounded by other variables. However, previous studies have shown both positive and negative results regarding oxidatively damaged DNA induced in workers occupationally exposed to coke oven emissions. Zang et al. (33) measured leukocyte 8-oxodG levels in workers and found that high-exposed workers had even lower levels of 8-oxodG than low-exposed workers. Marczynski et al. (7) and Liu et al. (5) reported significantly higher 8-oxodG in WBC and urine, respectively, for workers exposed to coke oven emissions, although the correlations between 8-oxodG and urinary 1-OHP were missed in their studies. Wu et al. (26) showed significantly higher levels of urinary 8-oxodG in high-exposed workers and found that the individual levels of 8-oxodG were correlated with urinary 1-OHP concentrations. One possible reason for such a discrepancy may be the use of a single measurement in previous studies. As mentioned earlier, most studies were conducted by collecting one spot urine sample from each worker mostly at the end of the work week. However, the single measurement may miss information of interest because the compared biomarker of exposure (i.e., urinary 1-OHP) and the marker of effect (i.e., 8-oxodG) are parameters of different time slots (7). Urine sampling at the end of the work week has been widely used for the coke oven workers because urinary 1-OHP excretion levels were found to be increased during the course of a work week and its half-life ranges from 6 to 35 hours (37). Indeed, our study also found that urinary level of 1-OHP reached the top at the end of the work week (Fig. 2A, D6-Post). However, this trend was not found for urinary 8-oxodG. As shown in Fig. 2B, the urinary 8-oxodG at the end of work week had relatively lower levels compared with the other working days, probably because the biomarker of effect (e.g., 8-oxodG) may not only reflect the exposure during a week-shift but also a much longer period of exposure (43). Subsequently, the absence of significant correlation between 1-OHP and 8-oxodG in published studies could be also explained by that, in addition to polycyclic aromatic hydrocarbons, coke oven emissions also contain a variety of nitrosamines, coal tar, metal compounds, and so forth. These co-contaminants derived from coke oven emissions may also contribute to the generation of reactive oxygen species and result in the change of urinary levels of 8-oxodG.

Although the chemical analysis of coke oven emissions has revealed the presence of nitrosamines (4), little work has been done to assess its potential role in DNA methylation induced by coke oven emissions. In this study, we measured urinary N7-MeG as an exposure biomarker of methylating agent among the coke oven workers. The results showed that the topside oven workers had significantly higher levels of urinary N7-MeG than side oven workers. The results showed that the topside oven workers had significantly higher levels of urinary N7-MeG than side oven workers (Table 2). Moreover, the urinary levels of N7-MeG were also found to be highly correlated with urinary 1-OHP levels for coke oven workers that were not confounded by other variables. These findings apparently suggest that coke oven emission exposure resulted in increased DNA methylation. To our best knowledge, it is the first study showing that occupational exposure to coke oven emissions could
induce DNA methylation by measuring the level of urinary N7-MeG.

Urinary N7-MeG has been previously measured in smokers for assessing the exposure of tobacco-specific nitrosamines. We previously measured urinary N7-MeG among smokers and nonsmokers using LC-MS/MS and reported the mean urinary levels of 4,215 and 3,035 ng/mg creatinine for smokers and nonsmokers, respectively (16). Ichiba et al. (44) also reported a mean urinary N7-MeG level of 4,600 ng/mg creatinine among smokers, a level that was found to be significantly decreased by 46% (2,500 ng/mg creatinine) after smoking cessation. As compared with the previous results, the topside oven workers in the present study had even higher urinary N7-MeG levels, ranging up to 6,904 ng/mg creatinine. Interestingly, the individual nitrosamine levels detected around the oven top area were substantially below the threshold of 1 µg/m³ (45); such low levels of nitrosamines, however, could not account for the formation of methylated DNA adducts observed in topside oven workers. Therefore, it could be reasonable to expect that there were other potential sources of methylating agents or their precursors (e.g., nitrogen oxides) resulting from coke combustion. Nitrogen oxides (i.e., NO and NO₂) are formed in all combustion processes, both from nitrogen in the fuel and from nitrogen in the air. Nevertheless, in the presence of amines, NO and NO₂ have been identified as the precursors of nitrosamines in vivo. Pitts et al. (46) and Iqbal et al. (47) both observed nitrosamine formation in mice after treatment with the secondary amines followed by exposure to NO₂ at concentrations as low as 0.3 and 0.2 ppm, respectively. In human study, Garland et al. (48) measured urinary excretion of nitrosodimethylamine in healthy subjects and found the mean urinary nitrosodimethylamine concentrations to be positively correlated with the ambient concentration of NO₂ ranging from 0.03 to 0.07 ppm. It has been reported that the concentration of nitrogen oxides detected was as high as 50 ppm around the oven top area in the coke oven plant (49). We suggested that this high concentration of nitrogen oxides might be able to contribute to an endogenous formation of nitrosamines and further resulted in a significantly increased DNA methylation for coke oven workers.

For over two decades, urinary mutagenicity has been widely applied in occupational and epidemiologic studies as a cost-effective and general biomarker of exposure to mutagenic compounds (18, 21). In this study, the results showed that the high-exposed workers had significantly higher mutagenicity than low-exposed workers. Moreover, this study further showed that the high-exposed workers had higher urinary acute toxicity compared with the low-exposed workers. These findings suggest that, in addition to urinary mutagenicity, acute toxicity of urine could also be a useful screening tool because it is capable of distinguishing different levels of coke oven emission exposure. Furthermore, increased urinary mutagenicity as well as the acute toxicity observed in this study, may strengthen epidemiologic evidence of the increased risk of renal and urinary tract tumors in these workers (1).

It is interesting that there was no obvious correlation between urinary mutagenicity and urinary 1-OHP concentration (P = 0.087), whereas the urinary acute toxicity was found to be slightly correlated with urinary 1-OHP (P = 0.042; Table 3). Although both urinary mutagenicity and 1-OHP concentrations have been used as biomarkers of exposure, the time course pattern of urinary mutagenicity was found to be different from that of urinary 1-OHP concentrations (Figs. 2A and 3B). This could be because urinary mutagenicity (and acute toxicity) provides an integrated measure of mutagenic/toxic exposure whereas 1-OHP has only served as a specific biomarker for polycyclic aromatic hydrocarbon exposure (21). Furthermore, it has been reported that the majority of urinary 1-OHP is conjugated to glucuronide (>80%) as a detoxification product of pyrene (50). Most urinary 1-OHP measurement was done after enzymatic deconjugation, in which a total 1-OHP was measured including both free-form and conjugated-form 1-OHP. However, urinary mutagenicity (or acute toxicity) was often conducted without enzymatic pretreatment to reflect a real toxicity in human urine, although previous studies have shown that the enzymatic deconjugation step could increase the mutagenicity and acute toxicity of urine (51, 52).

In conclusion, this is the first “repeated-measures” study of coke oven workers to assess potential adverse health burden related to the coke oven emission exposure using five different biomarkers, namely urinary 1-OHP, 8-oxodG, N7-MeG, acute toxicity, and mutagenicity. The results clearly showed that coke oven emission exposure could induce methylation and oxidative damage to DNA as well as increase the acute toxicity and mutagenicity in urine. More importantly, with the repeated measures in this study, a different pattern of time courses was observed for 8-oxodG, N7-MeG, acute toxicity, and mutagenicity in urine compared with that of urinary 1-OHP. This suggests that certain precautions should be taken when the single measurement is applied in a study because such sampling design could miss reading the information of interest. Furthermore, this study for the very first time suggests that, except for nitrosamines, other potential source of methylating agents and/or their precursors (i.e., NO₂) present in coke oven emissions could increase DNA methylation. This finding may raise questions about their role in coke oven emission–related carcinogenesis, and such questions wait to be explored.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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