Suboptimal DNA Repair Capacity Predisposes Coke-Oven Workers To Accumulate More Chromosomal Damages in Peripheral Lymphocytes

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Abstract

DNA repair is an essential mechanism for cells to maintain their genomic integrity under endogenous or exogenous assault. Reduced DNA repair capacity (DRC) is associated with increased risk for several environmentally related cancers. The micronucleus in peripheral lymphocytes has been validated as a biomarker of chromosomal damage, increasing cancer risk in human populations. We hypothesized that suboptimal DRC is associated with the increase in chromosomal damage among 94 coke-oven workers and 64 noncoke-oven controls. DRC was evaluated in isolated lymphocytes by comet assay. Chromosomal damage in peripheral lymphocytes was detected by cytokinesis-block micronucleus assay. Four common coding single nucleotide polymorphisms in the XRCC1 gene were genotyped. Coke-oven workers have significantly increased urinary 1-hydroxypyrene (9.0; 6.8-11.7 μg/L versus 1.5; 1.3-1.7 μg/L; P < 0.01) and micronucleus frequency (7.4% ± 4.3% versus 3.0% ± 3.0%; P < 0.01), and decreased DRC (55.9% ± 16.4% versus 63.6% ± 18.5%; P < 0.01) compared with controls. Significant correlations between DRC and micronucleus frequency were found in coke-oven workers (r = −0.32; P < 0.01; n = 94) and all study subjects (r = −0.32; P < 0.001; n = 158) but not in controls (r = −0.21; P = 0.11; n = 64). Variants of the Arg399Gln polymorphism were associated with a decreased DRC in both coke-oven workers (51.6% ± 16.1% versus 60.6% ± 15.7%; P < 0.01) and controls (59.1% ± 18.5% versus 68.4% ± 17.5%; P = 0.04). The complicated interrelationship of these multiple biomarkers was also identified by path analysis. These findings should facilitate developing a biomarker-based risk assessment model for lung cancer in this occupational population. (Cancer Epidemiol Biomarkers Prev 2009;18(3):987–93)

Introduction

DNA repair is an essential mechanism for cells to maintain their genomic integrity under endogenous or exogenous assault. DNA repair capacity (DRC) evaluated in in vitro short-term cultured lymphocytes has been validated to be a risk factor for lung cancer (1). Recently, Wu et al. (2) evaluated the heritability of DRC among 255 twins, indirectly measured by mutagen sensitivity assay to four different mutagens, and found that up to 40% to 60% of the variability of this phenotype can be explained by genetics, validating the use of DRC as a cancer susceptibility factor. The cytokinesis-block micronucleus (CBMN) assay in peripheral lymphocytes has been extensively studied as a biomarker of chromosomal damage and genome stability relevant to cancer risk in human populations (3). Recently, the Human MicroNucleus project summarized micronucleus data collected between 1980 and 2002 from 6,718 subjects from 10 countries and found that micronucleus frequency in peripheral lymphocytes is a strong predictive biomarker of cancer risk within healthy populations (4). Increased CBMN frequency was found in coke-oven workers exposed to high levels of coke-oven emissions, and genetic variants in several key DNA repair genes were found to be associated with this risk phenotype for cancers (5-7). Thus, DRC, as a phenotypic summary of multiple allelic variations in related biological pathways (8-10), could be a risk factor for chromosomal damage among coke-oven workers with a high risk for lung cancer (5).

X-ray cross-complementing group 1 protein (XRCCI) plays a key role in base excision repair, which repairs DNA strand breaks and damaged bases. Among the four common single nucleotide polymorphisms (SNP) in coding regions of the XRCC1 gene at codon 194 (Arg to Trp), 280 (Arg to His), 399 (Arg to Gln), and 632 (Gln to Gln), Gln632Gln is in complete linkage disequilibrium with −77 T/C at the 5′ untranslated region in the Han Chinese (6), and the −77 T/C SNP can influence the mRNA expression level of XRCC1 (11). These four SNPs...
have been extensively studied in relation to mutagen sensitivity and cancer risk, and many studies found a significant association between the Gln399 variant of the XRCC1 gene and decreased DRC and/or increased risk for cancers (12). In our previous study, variants in XRCC1 were associated with both DNA and chromosomal damage in coke-oven workers, suggesting that polymorphisms in XRCC1 may be a key modulator for the association between polycyclic aromatic hydrocarbon exposure and genetic damage among coke-oven workers (6, 13). Therefore, it is highly interesting to test whether SNPs in XRCC1 could influence bleomycin-induced DRC among coke-oven workers.

In this study, we hypothesized that DRC induced by bleomycin, which mainly induces DNA strand breaks and damages bases, is associated with chromosomal damage in peripheral lymphocytes among populations occupationally exposed to coke-oven emissions. This hypothesis was tested in 94 coke-oven workers and 64 noncoke-oven workers. The influence of XRCC1 polymorphisms and coke-oven exposure on DRC is also discussed. Path analysis revealed the interrelationship of the polycyclic aromatic hydrocarbon exposure, genetic status of XRCC1, DRC, and chromosomal damage.

Materials and Methods

Subjects and Sample Collection. This study was approved by the Research Ethic Committee of the National Institute for Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention. All study participants were employed at a steel company in Northeast China. The exposure group consisted of 94 workers from one coking plant who had been exposed to coke-oven emissions regularly and had been employed for at least 6 mo. Sixty-four noncoke-oven workers were recruited from an ore dressing plant that is located ~9 km from the coking plant. Workers from the ore dressing plant were selected as controls as they had no exposure to any obvious occupational DNA damaging agents, including coke-oven emissions, and were in the job category with overall the same salary and benefits as coke-oven workers. Exclusion criteria for participation in the study included recent treatments with mutagenic agents (such as X-rays), chronic conditions (such as autoimmune disease), recent acute infections that required medications such as antibiotics and refusal to sign the consent form. The participation rates for coke-oven workers and noncoke-oven controls were 100% and 98.5%, respectively. Most of the 158 study participants lived ~4 km away from the coking plant in a large residential area previously owned by the steel plant. All participants were interviewed by an occupational physician using a detailed questionnaire that included demographic information, educational level, smoking history, alcohol consumption, occupational history of exposure, and personal medical history. Individuals who had smoked >100 cigarettes in their lifetime were considered smokers. In this study, no smokers quit smoking before this interview. Individuals who drank more than twice a week in the last 6 mo were classified as alcohol users.

Spot urine samples were obtained from each subject at the end of a work shift after at least 4 consecutive days of work. Four milliliters of venous blood samples were drawn from each subject for the CBMN assay, DRC assay, and the extraction of genomic DNA.

Urinary 1-Hydroxyxpyrene Level as an Internal Biomarker of Exposure. The excretion of urinary 1-hydroxyxpyrene (1-OHP) was measured as the internal dose of personal, recent polycyclic aromatic hydrocarbon exposure (5, 14). Quality control samples were prepared by spiking a urine sample collected from a never-smoker with 1-OHP at the concentrations of 5 and 20 μg/L. Twelve spike urine samples with 1-OHP concentrations of either 5 or 20 μg/L were analyzed to establish the reference values before running the formal samples. Three pairs of spike urine controls were inserted into and analyzed with each batch of 20 formal urine samples. The 1-OHP measurements for each batch of samples were not accepted until the difference between the measured average level of 1-OHP for the quality control samples and the corresponding reference value was <10%. Three coke-oven workers and 14 noncoke-oven controls had 1-OHP measurements below the limit of detection. Measurements below the limit of detection were replaced with limit of detection / 2 before statistical analysis (15).

Chromosomal Damage in Peripheral Blood Lymphocytes. The CBMN assay was done according to the standard procedure as described previously (16). Duplicate lymphocyte cultures for each subject were set up. A total of 1,000 binucleated lymphocytes were examined for micronucleus detection for each subject. The CBMN frequency was estimated as the number of micronuclei per 1,000 binucleated lymphocytes. The scorer of the slides was blind to both the exposure status of study subjects and all other measurements investigated in this study.

DRC Assessment. Freshly isolated lymphocytes were cultured in RPMI 1640 supplemented with 2 mmol/L of L-glutamine, 20% FBS, 2% phytohemagglutinin, and 100 U/mL of penicillin and streptomycin. Cells were adjusted to 1 x 10⁵ cells/mL and incubated at 37°C in 5% CO₂.

The comet assay was modified to evaluate the removal kinetics of DNA damage induced by bleomycin as an indirect measurement for DRC (17-20). Freshly isolated lymphocytes from 4 healthy nonsmoking male volunteers (ages 30-40 y) were used to optimize the concentrations for bleomycin treatment and incubation time for DNA repair. A 30-min treatment of 8 μg/mL of bleomycin in serum-free medium was selected as the optimal dosing conditions because of lower cytotoxicity but significant genotoxicity (Supplementary Fig. S1A). The time course for repair was studied under 8 μg/mL bleomycin treatment, and 45 min of incubation in normal medium was found to be enough to show the maximum DNA repair in most of the cells (Supplementary Fig. S1B). The flow chart for the DRC assay is shown in Supplementary Fig. S1C. Slides were made just before bleomycin treatment, at the end of 30-min bleomycin treatment, and at the end of 45-min repair. Then the slides were processed for the comet assay.

The details of the comet assay were introduced in our previous paper (13). The freshly prepared slides were
fixed in ice-cold ethanol, air dried, and shipped back to the laboratory for analyzing the DNA damage. One hundred random selected cells were scored for each subject (50 cells per each of two replicate slides). Olive tail moment (TM) was used as measurement of DNA migration in this study. The arithmetic mean of the olive TM of 100 cells is presented as the average DNA damage level for each subject in the following statistical analysis. Three TMs were measured, including TM1 as TM of cells before bleomycin treatment, TM2 as TM of cells at the end of 30-min bleomycin treatment, and TM3 as TM at the end of 45-min repair. DRC was defined as the percentage of repaired DNA damage caused by bleomycin in the 45-min incubation period, which is calculated by the following equation, [(TM2 – TM3) / (TM2 – TM1)] × 100%. To control the cross-batch variation of DRC, lymphocytes from a healthy male donor were collected repeatedly and processed together with the samples from the study subjects. The average DRC in 6 samples from this person during 2 wk was 69.52 with a SD of 5.63 (range, 62.78-76.39).

Polymorphism Analysis of the XRCCI Gene. Four SNPs Arg194Trp (C26304T, rs1799782), Arg280His (G27466A, rs25789), Arg399Gln (G28152A, rs25487), and Gln632Gln (G36189A, rs3547) were detected by the published methods (21, 22). Ten percent of the DNA samples were genotyped for a second time and the concordance was 100%.

Statistical Analysis. The distribution of DRC was normal in both coke-oven workers and noncoke-oven controls. Urinary 1-OHP and CBMN frequency followed an approximately log-normal distribution, and thus, their ln-transformed values were used in the analysis. The arithmetic mean of the olive TM of 100 cells is presented as the average DNA damage level for each subject in the following statistical analysis. Three TMs were measured, including TM1 as TM of cells before bleomycin treatment, TM2 as TM of cells at the end of 30-min bleomycin treatment, and TM3 as TM at the end of 45-min repair. DRC was defined as the percentage of repaired DNA damage caused by bleomycin in the 45-min incubation period, which is calculated by the following equation, [(TM2 – TM3) / (TM2 – TM1)] × 100%. To control the cross-batch variation of DRC, lymphocytes from a healthy male donor were collected repeatedly and processed together with the samples from the study subjects. The average DRC in 6 samples from this person during 2 wk was 69.52 with a SD of 5.63 (range, 62.78-76.39).

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### Table 1. Demographics and biomarkers of coke-oven workers and noncoke-oven controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>Noncoke-oven Controls</th>
<th>Coke-oven workers</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>64</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>Age (y, mean ± SD)</td>
<td>41.8 ± 4.7</td>
<td>41.2 ± 6.8</td>
<td>0.51*</td>
</tr>
<tr>
<td>Gender (men/women, % of men)</td>
<td>64/0 [100]</td>
<td>92/2 [98]</td>
<td>0.51*</td>
</tr>
<tr>
<td>Current smokers (yes/no, %)</td>
<td>47/17 [73]</td>
<td>73/21 [78]</td>
<td>0.57</td>
</tr>
<tr>
<td>Alcohol user (yes/no, %)</td>
<td>38/26 (69.4)</td>
<td>69/25 [73.4]</td>
<td>0.08</td>
</tr>
<tr>
<td>Coking history (y, mean ± SD)</td>
<td>20.7 ± 7.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Urinary 1-OHP (µg/L, GM 95% CI)</td>
<td>1.5 (1.3-1.7)</td>
<td>9.0 (6.8-11.7)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CBMN frequency (% mean ± SD)</td>
<td>3.0 ± 3.0</td>
<td>7.4 ± 4.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Baseline DNA damage (GM 95% CI)</td>
<td>0.43 (0.35-0.52)</td>
<td>0.86 (0.77-0.97)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Minor allele frequency of XRCCI polymorphisms (%)</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>T of C26304T (Arg194Trp)</td>
<td>31</td>
<td>26</td>
<td>0.31</td>
</tr>
<tr>
<td>A of G27466A (Arg280His)</td>
<td>8</td>
<td>9</td>
<td>0.70</td>
</tr>
<tr>
<td>A of G28152A (Arg399Gln)</td>
<td>28</td>
<td>32</td>
<td>0.47</td>
</tr>
<tr>
<td>A of G36189A (Gln632Gln)</td>
<td>8</td>
<td>6</td>
<td>0.49</td>
</tr>
<tr>
<td>Educational level (%)</td>
<td>—</td>
<td>—</td>
<td>0.62</td>
</tr>
<tr>
<td>Middle school</td>
<td>7</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>High school or equivalent</td>
<td>18</td>
<td>20</td>
<td>—</td>
</tr>
<tr>
<td>College or above</td>
<td>75</td>
<td>70</td>
<td>—</td>
</tr>
</tbody>
</table>

*Two-sided two-sample t test between coke-oven workers and noncoke-oven controls.
†χ² tests for differences between coke-oven workers and noncoke-oven controls.
‡Two-sided two-sample t test for ln-transformed data between coke-oven workers and controls.
Vocational education was treated as high school equivalent.
Results

The demographic data and the levels of biomarkers for coke-oven workers and noncoke-oven controls are summarized in Table 1. The distributions of age, prevalence of smoking and drinking, XRCC1 genotypes, and educational level were not significantly different between the two groups. The median of the coking history among the coke-oven workers was 20.7 years. The geometric mean of urinary 1-OHP was significantly higher in coke-oven workers (9.0; 95% CI, 6.8-11.7 μg/L) than in controls (1.5; 95% CI, 1.3-1.7 μg/L). The average CBMN frequency was 7.4% ± 4.3% in coke-oven workers, which was significantly higher than that in controls (3.0% ± 3.0%). The basal DNA damage after 20 h of culture was 0.86 (95% CI, 0.77-0.97) among coke-oven workers, which was significantly higher than that in controls (0.43; 95% CI, 0.35-0.52), suggesting that some severe damage could not be repaired easily.

The allelic distributions for the four XRCC1 SNPs were in Hardy-Weinberg equilibrium either in all subjects or in coke-oven workers or in noncoke-oven controls (data not shown). Coke-oven workers have significantly reduced DRC compared with noncoke-oven controls (55.9% ± 16.4% versus 63.6% ± 18.5%; P < 0.01). The mean level of bleomycin-induced DRC remained lower in coke-oven workers than in noncoke-oven controls when subjects were stratified by age, smoking and drinking status, and XRCC1 genotypes (Supplementary Table S1). The odds ratio and 95% CIs were calculated to further characterize the association between the coke-oven exposure and DRC. We chose to dichotomize the percentage of DRC by using the median of DRC in noncoke-oven controls (65.9%). This allowed for an adequate overlap in the distribution of DRC in workers and controls. The risk for having suboptimal DRC (<65.9%) in coke-oven workers was 2.13 (95% CI, 1.11-4.10), with adjustment for selected covariates. This result did not differ significantly from other cut points.

The effects of XRCC1 genotypes on DRC are summarized in Supplementary Table S1. In coke-oven workers, a significant association between C28152A (Arg399Gln) and DRC was found. The DRC was significantly lower in subjects with the GA/AA (51.6% ± 16.1%) genotype compared with those with GG genotype (60.6% ± 15.7%; P < 0.01). This association was also observed in controls. The DRC in subjects with the GA/AA genotype (59.1% ± 18.5%) was significantly lower than in those with the GG genotype (68.4 ± 17.5%; P = 0.04). The associations between C26304T (Arg194Trp), G27466A (Arg280His), and G36189A (Gln632Gln) polymorphisms and DRC were not statistically significant in either coke-oven workers or controls. No association was found between age, smoking and drinking status, coking history, and DRC in this study population.

A significant correlation between DRC and ln-transformed CBMN frequency was found in the entire population (r = 0.32; P < 0.001) and in coke-oven workers (r = 0.32; P < 0.003), adjusting for age, smoking and drinking status, coking history among coke-oven workers, and urine 1-OHP (Supplementary Fig. S2). In controls, the correlation between DRC and ln-transformed CBMN did not reach statistical significance (r = 0.11) when adjusted for age, smoking and drinking status, and urine 1-OHP (Supplementary Fig. S2). No association between the XRCCI Arg399Gln genotype and CBMN frequency was found among the study population (data not shown). However, when the data were stratified by the XRCCI Arg399Gln

Table 2. Annotation of variables included in the path analysis

<table>
<thead>
<tr>
<th>Variables</th>
<th>Annotation</th>
<th>Value assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>LnOHP</td>
<td>Ln-transformed urinary 1-OHP</td>
<td>Numerical type</td>
</tr>
<tr>
<td>LnCBMN</td>
<td>Ln-transformed CBMN frequency</td>
<td>Numerical type</td>
</tr>
<tr>
<td>DRC</td>
<td>DRC</td>
<td>Numerical type</td>
</tr>
<tr>
<td>Coke work</td>
<td>Coke-oven worker or not</td>
<td>0, noncoke-oven controls; 1, coke-oven worker</td>
</tr>
<tr>
<td>Smoke</td>
<td>Smoking status</td>
<td>0, never smoker; 1, current smoker*</td>
</tr>
<tr>
<td>Age</td>
<td>Categories of age</td>
<td>0, ≤ 41 y; 1, &gt; 41 y</td>
</tr>
<tr>
<td>XRCC1399</td>
<td>Arg399Gln polymorphism</td>
<td>0, w/w; 1, m/w and m/m</td>
</tr>
</tbody>
</table>

*No former smokers were found in this study.

A significant correlation between DRC and ln-transformed CBMN frequency was found in the entire population (n = 158; r = 0.32; P < 0.001) and in coke-oven workers (n = 94; r = 0.32, P = 0.003), adjusting for age, smoking and drinking status, coking history among coke-oven workers, and urine 1-OHP (Supplementary Fig. S2). In controls, the correlation between DRC and ln-transformed CBMN did not reach statistical significance (n = 64; r = 0.21; P = 0.11) when adjusted for age, smoking and drinking status, and urine 1-OHP (Supplementary Fig. S2). No association between the XRCCI Arg399Gln genotype and CBMN frequency was found among the study population (data not shown). However, when the data were stratified by the XRCCI Arg399Gln
genotype, the correlation between DRC and CBMN frequency was more pronounced in coke-oven workers with the GG genotype \((n = 44; r = -0.47; P = 0.004)\) than in those with GA or AA \((n = 30; r = -0.15; P = 0.33;\) data not shown). The association between DRC and micronucleus frequency varying by the genotype of XRCC1 Arg399Gln polymorphism suggests that the variant genotype may be related to an impaired DRC, which is easily saturated.

The path analysis was used to identify the complicated causal interrelationship of various biomarkers measured in this study. The annotation of all the variables included in the path analysis is listed in Table 2. Indices used for the overall evaluation of the model were as follows: goodness-of-fit, 0.993; adjusted goodness-of-fit, 0.979; root mean square error of goodness-of-fit, 0.993; adjusted goodness-of-fit, 0.986; the overall evaluation of the model were as follows: 0.11, which showed that the variables included in this model had explained 36% of the total variance of urine 1-OHP. The XRCC1399 polymorphism and coke work were two significant determinants for DRC. Their importance reflected by the path diagram. Each arrow is accompanied by a path coefficient, and the widths of the arrows are drawn proportionally to the absolute magnitude of the corresponding path coefficients. Dashed lines, equal negative causal relation.

The effect decompositions for variables associated with either DRC or CBMN frequency are presented in Table 3. The interrelationship between multiple biomarkers measured in this study is further presented in Fig. 2. Coke work and the XRCC1399 polymorphism only directly affect the DRC. Coke work directly and indirectly affects CBMN frequency. The indirect effect of coke work on CBMN frequency is through the lnOHP and DRC paths, as shown in Fig. 2. Smoke indirectly affects CBMN frequency through the lnOHP path. XRCC1399 indirectly affects CBMN frequency through the DRC path. Age, lnOHP, and DRC all directly affect CBMN frequency.

**Discussion**

In this study, we found that bleomycin-induced DRC in peripheral lymphocytes is strongly associated with the micronucleus, a validated biomarker for the risk of several cancers (4), among coke-oven workers. These results support the hypothesis that subjects with suboptimal DRC may be more susceptible to accumulating mutations during chemical carcinogenesis. In studying the determinants for bleomycin-induced DRC, we found that both environmental exposure and genetic variants in the XRCC1 gene could work coordinately to influence the DRC.

Alkaline comet assay is used to measure the kinetics of DNA repair in peripheral lymphocytes after

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**Table 3. Standardized effect decompositions for DRC and CBMN frequency**

<table>
<thead>
<tr>
<th>Variables*</th>
<th>DRC</th>
<th>lnCBMN*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Direct effect</td>
<td>Indirect effect</td>
</tr>
<tr>
<td>Coke work</td>
<td>-0.211</td>
<td>0.000</td>
</tr>
<tr>
<td>XRCC1399</td>
<td>-0.259</td>
<td>0.000</td>
</tr>
<tr>
<td>Smoke</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Age</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>lnOHP</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>DRC</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*Variables are annotated in Table 2.
† The total effect for each variable is decomposed to direct effect and indirect effect. The direct effect measures the direct contribution of each variable to the end points. The indirect effect measures the contribution of each variable to the end points by their path of influencing other variables that have direct effect on the end points.
bleomycin treatment. In our assay, peripheral lymphocytes were stimulated by phytohemagglutinin in the medium for 20 hours to bring the resting cells into G1 phase. Thus, DRC most likely was activated in these dividing lymphocytes, allowing us to see the difference between DNA repair-proficient subjects from inefficient ones. Previous studies already have shown that the ability of mammalian cells to remove DNA damage is closely correlated with cell proliferation and the expression of DNA repair enzymes are cell cycle-dependent (25). Considering that the difference of DRC between coke-oven workers and noncoke-oven controls may be due to the different cycling kinetics for lymphocytes after phytohemagglutinin stimulation, we checked the cell cycle by flow cytometry in lymphocytes collected from 20 coke-oven workers and 20 controls. The results indicated that the proportion of cells in different cell cycles was not different between the two groups (data not shown), validating the treatment strategy for the DRC assay in this study. Furthermore, a 20-hour culture can also minimize the interindividual differences of any preexisting background DNA damage. In our present study, the baseline DNA damage (TM1, 0.67; 95% CI, 0.35-1.15) was much smaller than the DNA damage induced by bleomycin treatment (TM2, 17.36; 95% CI, 12.95-23.16), and we used the value of TM2 minus TM1 as the part of DNA damage induced by bleomycin to eliminate the influence of basal damage when evaluating DRC.

Coke-oven workers have a 12% reduction in DRC compared with noncoke-oven controls. Reduced DRC also has been reported in populations exposed to 1, 3-butadiene (26), pesticides (27), traffic emissions (28), and uranium (29). However, the mechanism underlying the chemically induced reduction in DRC has not yet been studied. Coke-oven emissions are complex mixtures of dusts, vapors, and gases that typically include polycyclic aromatic hydrocarbons, formaldehyde, acrolein, aliphatic aldehydes, ammonia, carbon monoxide, nitrogen oxides, phenol, cadmium, arsenic, and mercury. Some chemicals among those identified in the coke-oven emissions can damage the cellular macromolecules, which may involve the genes and proteins required for repairing the DNA damage induced by coke-oven emissions. For example, polycyclic aromatic hydrocarbons are metabolized by human cells to form electrophilic derivatives, which bond covalently to nucleophilic residues in cellular macromolecules (30). Therefore, the DNA repair system in coke-oven workers could be overwhelmed or saturated when exposed to mutagen in vitro.

Age is another important factor for DRC. Wei et al. (31) observed an age-related decline in DRC, assessed by host cell reaction assay, which amounted to an ~0.61% decline per year that occurred in a normal population of ages 20 to 60 years. Recently, Leng et al. (32) found that age is well-correlated with mutagen sensitivity induced by bleomycin in a smoker cohort of ages 40 to 75 years. However, in the present study, we did not observe any effect of age on DRC, possibly because of a narrower age range in the study population (ages 30-50 years).

The results of path analysis revealed that coke-oven exposure was the most influential factor for the levels of urinary 1-OHP and CBMN frequency, which was consistent with our previous findings (23). The XRCC1 Arg399Gln polymorphism is the most influential factor for DRC, which has a greater path coefficient than that of coke work. Our results provided the direct evidence that intrinsic susceptibility and external exposure may be equally important in determining a specific DNA repair phenotype. Furthermore, path analysis did show that the germline XRCC1 Arg399Gln polymorphism had an effect on CBMN frequency by the path of DRC, supporting the potential causal relationship between suboptimal DRC and an increase in chromosomal damage among coke-oven workers. This result confirms our previous findings that genetic variation in XRCC1 is a key factor in modulating the DNA damage response in coke-oven workers (6, 13). Among coke-oven workers, age is the only factor identified influencing the CBMN frequency. Coke-oven workers aged >41 years have significantly higher CBMN frequency than those aged ≤41 years (8.37% ± 4.44% versus 5.78% ± 3.63%; P < 0.01). However, age is not associated with DRC in this study (P = 0.57). This result excludes the possibility that the association between DRC and CBMN frequency among coke-oven workers is just a coincidence related to age. This study also has several limitations. First, the DRC and micronucleus were all evaluated in peripheral lymphocytes but not bronchial epithelial cells. Therefore, whether the association between a reduced DRC and increased risk for chromosomal damage in lymphocytes could reflect the real situation in target cells needs to be validated by using bronchoscopy collected from these workers. A high correlation has been established between lymphocytes and bronchial epithelial cells for DNA-dependent protein kinase activity (33) and aromatic DNA adducts level (34), providing a mechanistic basis for peripheral lymphocytes to have a similar DNA repair kinetics as those of bronchial epithelial cells undergoing carcinogen treatment. Second, only a small portion of the variability was described by the pathway network analysis; thus, other unstudied risk factors need to be investigated. For example, DRC induced by bleomycin reflects the combined effects of many SNPs in genes in related pathways, such as base excision repair, double-strand break repair, and related cell cycles. Therefore, more functional SNPs in other genes need to be investigated in the future to increase the fit of the model. Third, the relatively small sample size in this study made us cautious in extrapolating our results.

In summary, our molecular epidemiologic study suggested that suboptimal DRC predisposes coke-oven workers to accumulate more chromosomal damage in peripheral lymphocytes. Furthermore, the path model in this report illuminated that both coke-oven emission exposure and genotypes of XRCC1 Arg399Gln are equally important in determining this DNA repair phenotype. These findings contribute to our understanding of the mechanism of lung carcinogenesis and should facilitate the eventual development of a lung cancer risk assessment model that includes environmental and occupational exposure, life-style risk factors, and informative biomarkers in this occupational population.

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