Association of Polymorphisms in AhR, CYP1A1, GSTM1, and GSTT1 Genes with Levels of DNA Damage in Peripheral Blood Lymphocytes among Coke-Oven Workers

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Abstract

Accumulating evidence has shown that both DNA damage caused by the metabolites of polycyclic aromatic hydrocarbons (PAH) and genetic polymorphisms in PAH-metabolic genes contribute to individual susceptibility to PAH-induced carcinogenesis. However, the functional relevance of genetic polymorphisms in PAH-metabolic genes in exposed individuals is still unclear. In this study of 240 coke-oven workers (the exposed group) and 123 non–coke-oven workers (the control group), we genotyped for polymorphisms in the AhR, CYP1A1, GSTM1, and GSTT1 genes by PCR methods, and determined the levels of DNA damage in peripheral blood lymphocytes using the alkaline comet assay. We found that the ln-transformed Olive tail moment (Olive TM) values in the exposed group were significantly higher than those in the control group (P < 0.001). Furthermore, in the exposed group, the Olive TM values in subjects with the AhR Lys<sup>554</sup> variant genotype were higher than those with the AhR Arg<sup>554</sup>/Arg<sup>554</sup> genotype (P = 0.021). Similarly, the Olive TM values in the non–coke-oven workers with the CYP1A1 MspI CC + CT genotype were lower than the values of those with the CYP1A1 MspI TT genotype (P = 0.005). However, these differences were not evident for GSTM1 and GSTT1. These results suggested that the polymorphism of AhR might modulate the effects of PAHs in the exposed group; however, the underlying molecular mechanisms by which this polymorphism may have affected the levels of PAH-induced DNA damage warrant further investigation.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a well-established group of chemical carcinogens (1). PAHs are formed and released during the coking process, resulting in the direct exposure of coke-oven workers to PAHs by inhalation and dermal contact (2). Epidemiologic studies indicate that the risks of some cancers are higher in workers exposed to the coking process compared with those who were not (3, 4).

The toxicity of PAHs, such as benzo(a)pyrene and benzo(a)anthracene, is mediated by aryl hydrocarbon receptors (AhR), which are ligand-activated transcriptional factors that play an important role in benzo(a)pyrene-induced carcinogenesis (5). After binding with benzo(a)pyrene, AhR translocates into the nucleus to interact with xenobiotic responsive elements, resulting in the up-regulation of phase I and II enzymes, such as cytochrome P450 and glutathione S-transferases, which are also involved in the metabolism of PAHs. It is thought that it is DNA damage, caused by reactive oxygen species formed during the metabolism of PAHs, that plays a role in PAH-induced carcinogenesis (6-8). Furthermore, the DNA damage caused by PAHs in coke-oven workers could be detected in their peripheral primary lymphocytes using the alkaline comet assay (9, 10), whereas PAH-induced carcinogenesis could be modulated by genetic variation (11, 12).

One of the polymorphisms in human AhR, G1661A (Arg<sup>554</sup>Lys; rs2066853), was first discovered by Kawajiri et al. (13), and has been the most commonly studied polymorphism in the AhR gene ever since. However, no study has investigated the effect(s) of AhR polymorphisms on the levels of DNA damage among coke-oven workers.

Cytochrome P450 1A1 (CYP1A1), a member of the phase I enzymes, is involved in the metabolic activation of PAHs, and a MspI RFLP in this gene was reported to be associated with the risk of lung cancer (11, 14). In coke-oven workers, the effect of this polymorphism on PAH-induced DNA damage was unclear because this polymorphism was also reported to be associated with a significant increase of urinary 1-hydroxypyrene concentrations (15), but not with increased tail extent moment in lymphocytes (16).

Glutathione S-transferases are members of the phase II enzymes, a multigene family. The glutathione S-transferase family includes five subgroups (i.e., α, π, μ, τ, and θ), and GSTM1 or GSTT1 alleles are lost in a subset of the general population (17). Previous studies indicated that individuals null for GSTM1 or GSTT1 genes seemed to be susceptible to some cancers, especially lung and bladder cancers (18-20). However, the results thus far are inconsistent for the effects of glutathione S-transferase polymorphisms on exposure biomarkers in coke-oven workers. For instance, the GSTM1 null genotype was found to be associated with increased levels of benzo(a)pyrene diol epoxide–DNA adducts among the coke-oven workers (21, 22), but this association was not confirmed by other studies (23-25).
We have previously reported that the levels of DNA damage in coke-oven workers were higher than in those who had no history of PAH exposure (26), but the establishment of the role of genetic polymorphisms of the PAH-metabolic genes in the levels of DNA damage in exposed workers may provide the rationale for occupational surveillance for the individuals susceptible to PAH-induced carcinogenesis. Therefore, we are interested in whether or not these genetic polymorphisms could modulate the levels of PAH-induced DNA damage in these coke-oven workers. In the present study, we investigated the association of some select genetic polymorphisms of AhR, CYP1A1, GSTT1, and GSTM1 genes with the levels of DNA damage in coke-oven workers.

Materials and Methods

Study Subjects. This study included 240 coke-oven workers and 123 non–coke-oven workers, who were all males, and worked in the same steel company in northern China. These 240 coke-oven workers were in active service at the time of the study, were employed for at least 6 months, and were recruited as the exposed group. These coke-oven workers worked on different work sites: top-oven, side-oven, and bottom-oven. The 123 non–coke-oven workers were staff members of the offices and hospitals of the same steel company, and served as the control group. The workers exposed to known mutagenic agents, such as radiotherapy and chemotherapy in the last 3 months, were excluded. After having obtained informed consent from each subject, we administered a standardized occupational questionnaire for each participant to collect information on demographic information, smoking and alcohol habits, occupational exposure, and medical and personal histories. In the morning, fasting venous blood was collected in 5 mL heparinized tubes and stored at 4°C. The genotypes of AhR, CYP1A1, GSTT1, and GSTM1 genes with the levels of DNA damage in coke-oven workers were determined by a multiplex PCR as previously described (28, 29). Briefly, the absence of a GSTM1-specific 230-bp or GSTT1-specific 480-bp product indicated the GSTM1-null or the GSTT1-null genotype when the positive control of β-actin-specific 157-bp product was present detected simultaneously in one reaction. TheMspI polymorphism in the CYP1A1 gene was identified by PCR-RFLP (15). The uncut PCR product of 340 bp was the T allele, and the C allele was cut, showing two smaller products of 200 and 140 bp. Analysis of the G1661A (Arg<sup>554</sup> Lys) polymorphism of the AhR gene was conducted according to the published protocol (30). Briefly, the AhR allele-specific primers were used to produce a 333 bp product. Amplification of a 493-bp product of the β-actin gene was used as an internal control. For quality control, 10% of DNA samples were genotyped again and the concordance of two sets of genotyping data was 100%.

Comet Assay. Lymphocytes were isolated from 1 mL of heparinized whole blood from the subjects and suspended in 1,000 μL ice-cold PBS (pH 7.4). The Comet assay was done according to the published protocol with a minor modification (31). Briefly, 100 μL of low-melting agarose [1% (v/v) in PBS (pH 7.4)] at 37°C mixed with 10 μL of PBS containing lymphocytes was transferred onto a precoated [0.5% (v/v) normal melting agarose in PBS (pH 7.4)] slide. Electrophoresis was conducted for 20 minutes at 25 V. DNA damage was measured using an image analysis system (version 1.0, IM Comet Analysis Software, China; ref. 32). Fifty cells were analyzed per slide, and the Olive tail moment (Olive TM) value was used as a measurement of DNA damage level as recommended (31, 32).

Statistical Analyses. All data analyses were carried out using the statistical analysis software SPSS 12.0 package. Olive TM values were normalized by natural logarithm (ln) transformation. The frequencies of categorical variables, such as smoking and drinking status between groups were compared by χ² tests. Student’s t tests were used to compare the ln-transformed Olive TM values followed by stratification according to smoking, alcohol use, and other groups. The Mann-Whitney U test was used to compare air borne PAHs and pack-years of cigarette-smoking between the two groups. The associations between the AhR, CYP1A1, GSTT1, and GSTM1 genotypes and the ln-transformed Olive TM values were tested by analysis of covariance, followed by a Bonferroni correction for multiple comparisons with adjustment for age, work site, and pack-years of cigarette smoking.

Results

General Characteristics and Olive TM. As shown in Table 1, no significant difference was found in the distributions of age, smoking status, and drinking status between the coke-oven workers and the non–coke-oven workers. However, the median pack-years of cigarette smoking in the coke-oven

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group (n = 123)</th>
<th>Exposed group (n = 240)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y, mean ± SD)</td>
<td>37.1 ± 4.9</td>
<td>37.1 ± 6.2</td>
<td>0.891*</td>
</tr>
<tr>
<td>Smoking status [yes/no (%)]</td>
<td>87/36 (70.7)</td>
<td>190/50 (79.2)</td>
<td>0.072</td>
</tr>
<tr>
<td>Pack-years smoked [median (range)]</td>
<td>8.7 (0-58.9)</td>
<td>13.4 (0-66.8)</td>
<td>0.006*</td>
</tr>
<tr>
<td>Drinking [yes/no (%)]</td>
<td>55/68 (44.2)</td>
<td>118/122 (49.2)</td>
<td>0.421</td>
</tr>
<tr>
<td>Olive TM (mean ± SD)</td>
<td>0.58 ± 0.92</td>
<td>1.23 ± 1.12</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Sum of carcinogenic PAHs [μg/m³, median (range)]</td>
<td>0.306 (0.233-0.350)</td>
<td>0.852 (0.259-3.330)</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*Student’s t tests for comparison between two groups. 

†Mann-Whitney U tests for comparison between two groups.

Table 1. Characteristics of the subjects by exposure status (n = 363)


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workers (13.4) were significantly higher than that of the non-coke-oven workers (8.7; P = 0.006). The Olive TM values in the coke-oven workers were significantly higher compared with the non-coke-oven workers (1.23 ± 1.12 versus 0.58 ± 0.92; P < 0.001). The median sum of eight carcinogenic PAHs was higher in the exposed group (0.852 μg/m³) than in the control group (0.306 μg/m³; P < 0.001). The median sums of eight carcinogenic PAHs at different work sites varied within the exposed group, but the differences were not statistically significant (data not shown).

To evaluate the effects of smoking and drinking on DNA damage, the data on levels of DNA damage was further stratified by smoking and drinking status. The results suggested that no significant associations were found between the Olive TM values and the smoking and drinking status within both the exposed and control groups (Table 2), suggesting that the observed difference in the levels of DNA damage between the exposed and control groups was likely due to exposure to PAHs rather than smoking and drinking status in these coke-oven workers.

**Olive TM and Genotypes.** In all subjects, the AhR G1661A genotype and CYP1A1 MspI genotype distributions were in Hardy-Weinberg equilibrium (data not shown), but this could not be assessed for the GSTT1 and GSTM1 genotypes. However, the distributions of AhR, CYP1A1, GSTT1, and GSTM1 genotypes were not statistically different between the two groups (data not shown).

The analyses for associations between AhR, CYP1A1, GSTT1, and GSTM1 genotypes and the Olive TM values are summarized in Table 3 and Fig. 1. In the exposed group, the Olive TM values in subjects with AhR Lys554 variant genotype (1.37 ± 1.10) were significantly higher than that of those with the AhR Arg554/Arg554 genotype (1.02 ± 1.12, P = 0.021) after adjustment for age, work site, and pack-years of cigarette smoking. In the control group, the Olive TM values in subjects with the CYP1A1 MspI CC + CT genotype (0.32 ± 0.96) were lower than those with CYP1A1 MspI TT genotype (0.90 ± 0.98; P = 0.005). No associations between the AhR, GSTT1, and GSTM1 genotypes and the Olive TM values were found in the control group, nor was an association between the CYP1A1 genotypes and the Olive TM values found in the exposed group.

**Discussion**

In this study, we found that the Olive TM values among the coke-oven workers were significantly higher than that among the non-coke-oven workers. Although the pack-years of cigarette smoking were significantly higher in the exposed group than in the control group, our results clearly showed that the difference in the DNA damage levels between the two groups was due to exposure to coke-oven emission, independent of smoking effects. This result was in agreement with most of the previously published studies (9, 10, 16). Although this study provided some new insight into the role of genetic polymorphisms in select PAH-metabolic genes in the formation of PAH-induced DNA damage in the coke-oven workers, the results are limited due to the relatively small sample size and the lack of data on other sources of PAHs, such as diet and passive smoking.

It is intriguing that the AhR Lys554 variant allele was associated with increased Olive TM values only in the exposed group. Although the potential mechanism for such an effect on DNA damage in coke-oven workers remains unclear, it is likely that the exposure level in the exposed group might have been greater than the threshold of the capacity of the variant enzyme, whereas the exposure level in the control group might not, but this hypothesis needs to be further tested in future studies. This polymorphism is located in exon 10 of the AhR gene, which encompasses most of the transactivation domain (33). Hence, this polymorphism might be associated with the up-regulation of the expression of downstream genes, such as the CYP1A1 gene. For example, among nonsmoking subjects, this AhR polymorphism was associated with increased

### Table 3. Comparisons of the Olive TM values in subgroups stratified by genotypes of AhR, CYP1A1, GSTT1, and GSTM1 in the control and exposed groups

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control group</th>
<th>Exposed group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (mean ± SD)</td>
<td>P</td>
</tr>
<tr>
<td>AhR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>47 (0.57 ± 1.00)</td>
<td>Reference*</td>
</tr>
<tr>
<td>Lys/Arg</td>
<td>58 (0.67 ± 0.81)</td>
<td>0.621</td>
</tr>
<tr>
<td>Lys/Lys</td>
<td>18 (0.31 ± 1.52)</td>
<td>0.363</td>
</tr>
<tr>
<td>Lys/Arg-Lys/Lys</td>
<td>76 (0.59 ± 1.02)</td>
<td>0.942</td>
</tr>
<tr>
<td>CYP1A1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>47 (0.90 ± 0.98)</td>
<td>Reference*</td>
</tr>
<tr>
<td>CT</td>
<td>54 (0.42 ± 0.92)</td>
<td>0.012</td>
</tr>
<tr>
<td>CC</td>
<td>22 (0.11 ± 1.04)</td>
<td>0.002</td>
</tr>
<tr>
<td>CC + CT</td>
<td>76 (0.32 ± 0.96)</td>
<td>0.005</td>
</tr>
<tr>
<td>GSTT1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-null</td>
<td>59 (0.63 ± 0.98)</td>
<td>Reference*</td>
</tr>
<tr>
<td>Null</td>
<td>64 (0.53 ± 0.97)</td>
<td>0.935</td>
</tr>
<tr>
<td>GSTM1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-null</td>
<td>46 (0.54 ± 0.92)</td>
<td>Reference*</td>
</tr>
<tr>
<td>Null</td>
<td>77 (0.57 ± 1.00)</td>
<td>0.711</td>
</tr>
</tbody>
</table>

*Multiple analysis of covariance for comparisons of ln-transformed Olive TM values in subjects with various genotypes in the two groups after adjustment for age, work site, and pack-years of cigarettes smoking.
ethoxyresorufin-O-deethylase activity in peripheral blood lymphocytes in vitro exposed to 3-methylcholanthrene (34). In a Caucasian population, induced CYP1A1 activity was found to be higher in subjects with the \textit{AhR} Lys554 variant genotype than those with \textit{AhR} Arg554/Arg554 genotype (35). Furthermore, this polymorphism was also found to be associated with significantly increased CYP1A1 activity in healthy young women smokers (33), and adversely associated with the survival of individuals with soft tissue sarcoma (36). However, there are also some conflicting results about the phenotypic effects of this polymorphism. For example, Wong et al. found that the Lys554 allele was as efficient as the Arg554 allele in stimulating CYP1A1 mRNA expression in their in vitro expression system (37). Our findings suggest that this polymorphism might be functional and responsive when individuals were exposed to higher levels of PAHs because no association was found between this polymorphism and DNA damage in the non–coke-oven workers.

Another possibility is that our study lacked the power to reveal the effect of \textit{GSTM1} and \textit{GSTT1} polymorphisms on DNA damage in the exposed group, or that these enzymes have a tissue specificity for the target organs in which carcinogenesis may occur.

An unexpected finding in our study was that the Olive TM values in subjects with the CYP1A1 MspI CC + CT genotype were lower than those with the CYP1A1 MspI TT genotype in the non–coke-oven workers. This result was not in agreement with previous studies in which the CYP1A1 MspI CC + CT genotype was associated with high levels of urinary 1-hydroxypyrene among smokers with no occupational exposure to PAHs (after adjusting for age, ethnicity, and number of cigarettes per day; ref. 41) in the coke-oven workers (15). CYP1A1 is well known as a member of the phase I enzymes that are involved not only in the metabolic activation of PAHs, but also in the metabolism of other xenobiotics. The CYP1A1 MspI polymorphism is thought to be associated with the high inducibility of the gene in response to exposure to its substrates. Therefore, it is possible that the non–coke-oven workers might have been exposed to other kinds of substances that might need CYP1A1 to metabolize, but this hypothesis needs to be further tested in future studies.

In summary, this is the first report to show that polymorphism in \textit{AhR} is associated with the levels of DNA damage in peripheral blood lymphocytes among the coke-oven workers, suggesting that this polymorphism may modulate the effects of PAH exposure in occupational settings. However, the underlying mechanisms of this observed effect modification, and their related consequences, remain to be further investigated before this finding can be applied to monitoring individuals susceptible to the PAH-induced carcinogenesis.
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