Analyses of bronchial bulky DNA adduct levels and CYP2C9, GSTP1 and NQO1 genotypes in a Hungarian study population with pulmonary diseases

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Carcinogen–DNA adducts may represent an intermediate end-point in the carcinogenic cascade and may reflect exposure to chemical carcinogens, as well as susceptibility and, ultimately, cancer risk. Interindividual variability in activity of enzymes involved in the metabolism of polycyclic aromatic hydrocarbons to mutagenic diol epoxides may predict adduct levels and, indirectly, lung cancer risk. Using 32P-postlabeling methods, the levels of bulky DNA adducts were determined in macroscopically normal bronchial tissues obtained from resected lobes of 143 Hungarian patients with lung malignancy and other pulmonary conditions. DNA from normal tissue was also evaluated for polymorphisms in cytochrome P450 (CYPs) (e.g. CYP1A1, CYP1A2, CYP2C9 and CYP3A4) and GSTP1 forms, indicating that metabolic activation of tobacco smoke carcinogens by these CYPs is an important determinant of genotoxicity (12). Forms of human glutathione S-transferase (GST) are known to catalyze the detoxification of 2-alkylarylamine metabolites of CYPs (13). Among GST forms expressed in human tissues, GSTP1 has been shown to be expressed in the highest abundance in lung (13). NAD(P)H:quinone oxidoreductase (encoded by NQO1 or DIA4) is known to catalyze the reduction of the quinone moiety to a hydroquinone to reduce the production of oxygen radicals (14) and has been shown to prevent the formation of 2-alkylarylamine–DNA adducts (15). Genetic polymorphisms have been identified in the CYP1A1 (16,17), CYP2C9 (18), GSTP1 (19) and NQO1 (20) genes. These genetic polymorphisms are associated with amino acid substitutions in open reading frames or in flanking regions that may affect catalytic properties and/or expressed levels of those enzymes. The amino acid substitutions in the observed genetic polymorphisms of CYP2C9, GSTP1 and NQO1 have been reported respectively as follows: Arg144Cys and Ile359Leu (18), Ile105Val (19) and Pro187Ser (20). A polymorphism at codon 105 in the GSTP1 gene was recently found to be associated with susceptibility to bladder, testicular and lung cancer (19,24). Though not statistically significant, slightly increased and decreased lung cancer risk was observed with the CYP2C9*2 and CYP2C9*3 alleles, respectively, in a population in Los Angeles County, California (25,26). Little is known of the association of genetic polymorphisms of NQO1 with carcinogen–DNA adducts and related susceptibility to malignant diseases, except

Abbreviations: anti-BPDE, anti-benz[a]pyrene 7,8-diol 9,10-epoxide; B[a]P, benzo[a]pyrene; CYP, cytochrome P450; GST, glutathione S-transferase; NQO1, NAD(P)H:quinone oxidoreductase; PAH, polycyclic aromatic hydrocarbon.

Introduction

Carcinogens in cigarette smoke are clearly the major risk factors for human lung cancer, as documented in epidemiological studies (1–3). Exposure to tobacco smoke carcinogens, including polycyclic aromatic hydrocarbons (PAHs) and aromatic amines, may be monitored by measurement of PAH–serum albumin adducts (4) and 4-aminobiphenyl–hemoglobin adducts (5), respectively. Bulky DNA adducts have been measured in human lung and bronchial tissues by 32P-postlabeling in samples from cancer patients and have provided evidence of DNA damage caused by smoking (6,7).

Benzo[a]pyrene (B[a]P), a major carcinogenic component of tobacco smoke, is known to undergo metabolic activation to exert its carcinogenic effects. Moreover, formation of anti-BPDE–DNA adducts in human lung and colon mucosa by sensitive HPLC/fluorometric assays (8). Different forms of cytochromes P450 (CYPs) (e.g. CYP1A1, CYP1A2, CYP2C9 and CYP3A4) have been shown to catalyze the formation of an ultimate mutagenic and genotoxic diol epoxide of B[a]P (9–11). In a study using human laryngeal tissues, total DNA adduct levels correlated strongly with expressed levels of CYP1A1, CYP3A4 and CYP2C forms, indicating that metabolic activation of tobacco smoke carcinogens by these CYPs is an important determinant of genotoxicity (12). Forms of human glutathione S-transferase (GST) are known to catalyze the detoxification of B[a]P by conjugation of epoxide metabolites of B[a]P with glutathione (13). Among forms of GST expressed in human tissues, GSTP1 has been shown to be expressed in the highest abundance in lung (13). NAD(P)H:quinone oxidoreductase (encoded by NQO1 or DIA4) is known to catalyze the reduction of the quinone moiety to a hydroquinone to reduce the production of oxygen radicals (14) and has been shown to prevent the formation of B[a]P quinone–DNA adducts (15). Genetic polymorphisms have been identified in the CYP1A1 (16,17), CYP2C9 (18), GSTP1 (19) and NQO1 (20) genes. These genetic polymorphisms are associated with amino acid substitutions in open reading frames or in flanking regions that may affect catalytic properties and/or expressed levels of those enzymes. The amino acid substitutions in the observed genetic polymorphisms of CYP2C9, GSTP1 and NQO1 have been reported respectively as follows: Arg144Cys and Ile359Leu (18), Ile105Val (19) and Pro187Ser (20). A polymorphism at codon 105 in the GSTP1 gene was recently found to be associated with susceptibility to bladder, testicular and lung cancer (19,24). Though not statistically significant, slightly increased and decreased lung cancer risk was observed with the CYP2C9*2 and CYP2C9*3 alleles, respectively, in a population in Los Angeles County, California (25,26). Little is known of the association of genetic polymorphisms of NQO1 with carcinogen–DNA adducts and related susceptibility to malignant diseases, except
for the recent finding that the frequent allele in NQO1(Pro187) appeared to be a risk factor for lung cancer (27). In a previous study (28), the levels of adducts in a subset of this population were examined in relation to genetic polymorphisms in CYP1A1 and GSTM1. In that analysis, it was found that, although GSTM1 and CYP1A1MspI genotypes did not affect adduct levels, the GSTM1 genotype appeared to be a risk factor for squamous cell carcinoma. To our knowledge, associations between levels of BPDE adducts in the lung and genetic polymorphisms in CYP2C9, GSTP1 and NQO1 have not been evaluated. In the present study using additional samples, the relationship between DNA adduct levels in bronchial tissues from patients with pulmonary diseases and genetic polymorphisms in CYP2C9, GSTP1 and NQO1 was examined.

Materials and methods

Study population

Demographic characteristics of the study samples in which CYP2C9, GSTP1 and NQO1 genotypes were evaluated in relation to adducts are shown in Table I. The study population analyzed for both bronchial DNA adduct levels and CYP2C9, GSTP1 and NQO1 genotypes included 118 Hungarian patients with lung malignancy and 25 patients undergoing pulmonary surgery for other lung conditions, including abscesses, pulmonary actinomycosis, benign adenoma, aspergillosis, bronchectasis, pulmonary fibroma, pulmonary hamartoma, chronic pneumonitis, tuberculosis, hydatidoma, cystadenoma, arteriovenous fistula, lymphocytoma and stenosis. All of the surgical samples were from the resected lobes of patients and were macroscopically normal bronchial tissue distal to any suspected malignant or diseased tissue. The surgery was performed in the largest thoracic surgical center in Hungary and the samples were considered to be representative of the general population. Extraction of genomic DNA from the tissue samples was performed as described elsewhere (28).

Table I. Lung disease patients for which levels of bronchial DNA adducts and CYP2C9, GSTP1 and NQO1 genotypes have been determined

<table>
<thead>
<tr>
<th></th>
<th>Smokers (Sm, Ex-Sm)a</th>
<th>Non-smokers (Ex-Sm, N-Sm)b</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>n, total</td>
<td>104 (79, 25)</td>
<td>39 (19, 20)</td>
<td>143</td>
</tr>
<tr>
<td>Age range</td>
<td>22–69</td>
<td>34–74</td>
<td>22–74</td>
</tr>
<tr>
<td>Mean age ± SD</td>
<td>52.7 ± 9.2</td>
<td>54.5 ± 9.7</td>
<td>53.2 ± 9.4</td>
</tr>
<tr>
<td>n, male/female</td>
<td>83/21 (63/16, 20/5)</td>
<td>26/13 (16/3, 10/10)</td>
<td>109/34</td>
</tr>
</tbody>
</table>

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DNA adduct measurement

Bulk DNA adducts were determined by the 32P-postlabeling procedure as described previously (28) using nuclease P1 digestion of the normal nucleotides for sensitivity enhancement. The presence of excess ATP at the end of each reaction was verified and two to four measurements were taken for each sample. Assay variability was 25.4 ± 19.6%. The DNA adduct levels for two broad categories of smoking status were compared in relation to genotypes of CYP2C9, GSTP1 and NQO1. For that, subjects were categorized as ‘smokers’ (current smokers and recent ex-smokers, who stopped smoking <1 year before surgery) and ‘non-smokers’ (life-time non-smokers and those who stopped smoking >1 year before surgery) (Table I).

Determination of genotypes at the CYP2C9 locus

Two polymorphic sites (Arg144Cys and Ile359Leu) were measured in the DNA samples. Arg144Cys polymorphism was detected by a modification of the method of Furuya et al. (29). A CYP2C9-specific intron forward primer, 5′-TGATGTTGCGTGCTCTCAGA-3′, and a reverse primer corresponding to the 5′-end of CYP2C9 exon 3, 5′-ACCCTTTGTTTTCATGACTC-3′, were used. PCR amplification was performed in a reaction mixture (50 µl) which consisted of 10 mM Tris–HCl buffer (pH 8.3), 2% DMSO, 0.1% (v/v) Triton X-100, 50 mM KCI, 2.5 mM MgCl2, 200 µM dNTPs, 1 µM forward and reverse primers, 2.5 U Taq DNA polymerase (Promega) and 50 ng of genomic DNA. Reactions were carried out for 35 cycles at a heat-denaturing temperature of 94°C for 30 s, an annealing temperature of 58°C for 30 s and an elongation temperature of 72°C for 2 min in a Perkin Elmer DNA thermal cycler 9600. The PCR products were purified on a MicroSpin S-300 column (Pharmacia, Uppsala, Sweden) and digested with AvaII (New England Biolabs, Beverly, MA) and analyzed by 3% Metaphor agarose (FMC, Rockland, ME) gel electrophoresis. PCR products derived from CYP2C9Arg144 and CYP2C9Cys144 alleles were detected as DNA fragments of 259 and 57 bp or as a single DNA fragment of 316 bp, respectively. A PCR-based genotyping method was also developed for CYP2C9Ile359Leu using a forward (5′-TGACCGAGTCCAGAGATTC-3′) and a reverse primer (5′-AAA- CATGGGTTTCGAGTGAT-3′) as described by Wang et al. (30). Reaction mixtures for PCR amplification consisted of the same components as for the CYP2C9Arg144Cys polymorphism, except that 0.5 µM of the forward and reverse primers were used. PCR amplification was carried out as in the method for determining the CYP2C9Arg144Cys polymorphism, except for an annealing temperature of 66°C for 30 s. Restriction enzyme digestion was performed using NsiI (New England Biolabs) after purification of the PCR products on MicroSpin S-300 columns. PCR products derived from the CYP2C9Ile359 allele gave DNA fragments of 110 and 21 bp, whereas those derived from the CYP2C9Leu359 allele were not digested by NsiI and gave a single fragment of 131 bp.

Determination of genotype at the GSTP1 locus

Since two variant cDNAs encoding Ile and Val at codon 105 were isolated for GSTP1, the Ile105Val polymorphism was detected by a PCR-based genotyping method. The PCR reaction mixture (50 µl) consisted of 10 mM Tris–HCl buffer (pH 8.3), 2% DMSO, 1.5 mM MgCl2, 0.1% (v/v) Triton X-100, 50 mM KCI, 250 µM dNTPs, 1 µM forward (5′-ACCGACATCCCTTTCCCCCCTC-3′) and reverse (5′-TACCTGGCTTGTTGATTC-3′) primers, 2.5 U Taq polymerase and 50 ng of genomic DNA. PCR reactions were performed for 35 cycles at a heat-denaturing temperature of 94°C for 30 s, an annealing temperature of 66°C for 30 s and an elongation temperature of 72°C for 2 min. Subsequently, PCR products, purified on a Sepharose S-200 Spin column (Pharmacia), were treated with AvaII (New England Biolabs). Using this protocol, PCR products (440 bp) produced from a GSTP1Ile105 allele were not digested by AvaII, whereas those from the GSTP1Val105 allele gave DNA fragments of 213 and 227 bp after digestion with AvaII. Although Harries et al. recently reported a similar method for GSTP1 genotyping (19), different primer sets were used in order to yield larger PCR fragments with higher sensitivity and resolution against agar gel electrophoresis. Both methods were used in a subset of samples (n = 20), yielding identical GSTP1 genotypes.

Determination of genotypes at the NQO1 locus

The Pro187Ser polymorphism was also detected by PCR-based genotyping. The primers used were 5′-ATTCCTCTAGTGCCCGTAGG-3′ and 5′-AATCCTGCTGGAAGTTGAT-3′. The PCR reaction was performed in a mixture (50 µl) which consisted of 10 mM Tris–HCl buffer (pH 8.3), 2% DMSO, 2.5 mM MgCl2, 0.1% (v/v) Triton X-100, 50 mM KCI, 200 µM dNTPs, 1 µM forward and reverse primers, 2.5 U Taq polymerase and 50 ng of genomic DNA. PCR reactions were performed for 35 cycles at a heat-denaturing temperature of 94°C for 30 s, an annealing temperature of 66°C for 30 s and an elongation temperature of 72°C for 2 min. Restriction enzyme digestion of the PCR products was performed using HindII (New England Biolabs). The frequent allele (NQO1Pro187) was detected as one band with a length of 318 bp, whereas the infrequent allele was detected at 154 and 164 bp bands.

Statistical analysis

Comparison of DNA adduct levels between smokers and non-smokers and within genotypes was made using the Mann–Whitney U-test and analysis of variance.

Results

Bulk DNA adduct levels in human bronchial samples

Bulk DNA adducts were measured in relation to CYP2C9, GSTP1 and NQO1 genotypes from 143 individual bronchial
Table II. Levels of bronchial bulky DNA adducts in smokers and non-smokers with genotypes for CYP2C9, GSTP1 and NQO1 genes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Smokers (n)</th>
<th>Non-smokers (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9 Arg144Cys</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a/a</td>
<td>8.6 ± 4.4 (71)</td>
<td>5.7 ± 3.3 (26)</td>
</tr>
<tr>
<td>a/b</td>
<td>8.4 ± 3.7 (32)</td>
<td>5.2 ± 3.4 (13)</td>
</tr>
<tr>
<td>b/b</td>
<td>9.4 (1)</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.14</td>
<td>0.37</td>
</tr>
<tr>
<td>CYP2C9 Ile359Leu</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a/a</td>
<td>8.8 ± 4.3 (84)</td>
<td>5.8 ± 3.5 (32)</td>
</tr>
<tr>
<td>a/b</td>
<td>7.6 ± 3.5 (20)</td>
<td>4.5 ± 1.3 (7)</td>
</tr>
<tr>
<td>b/b</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.15</td>
<td>0.81</td>
</tr>
<tr>
<td>GSTP1 Ile105Val</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a/a</td>
<td>9.2 ± 3.7 (50)</td>
<td>5.4 ± 3.1 (19)</td>
</tr>
<tr>
<td>a/b</td>
<td>8.0 ± 4.6 (41)</td>
<td>6.0 ± 3.6 (17)</td>
</tr>
<tr>
<td>b/b</td>
<td>7.8 ± 4.6 (13)</td>
<td>4.0 ± 2.5 (3)</td>
</tr>
<tr>
<td>P</td>
<td>0.13</td>
<td>0.67</td>
</tr>
<tr>
<td>NQO1 Pro187Ser</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a/a</td>
<td>8.9 ± 4.3 (77)</td>
<td>5.7 ± 3.3 (35)</td>
</tr>
<tr>
<td>a/b</td>
<td>7.5 ± 3.3 (20)</td>
<td>4.9 ± 1.6 (3)</td>
</tr>
<tr>
<td>b/b</td>
<td>8.4 ± 5.4 (7)</td>
<td>1.4 (1)</td>
</tr>
<tr>
<td>P</td>
<td>0.10</td>
<td>0.88</td>
</tr>
<tr>
<td>All</td>
<td>8.6 ± 4.2 (104)</td>
<td>5.6 ± 3.3 (39)</td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

*Bronchial bulky DNA adduct levels were determined by 3P-post-labeling methods as described in Materials and methods and are expressed as the mean ± SD per 10^8 nucleotides. Genotypes for CYP2C9, GSTP1 and NQO1 were analyzed as described in Materials and methods. Numbers in parentheses represent the numbers of subjects. a and b are frequent and less frequent alleles, respectively. a/a and b/b represent homozygotes and a/b represents heterozygotes. Frequents alleles in this study population: Arg144 and Ile359 for CYP2C9; Ile105 for GSTP1; Pro187 for NQO1. Smokers: current smokers, who smoked up to the time of surgery and short-term ex-smokers who stopped smoking <1 year before surgery. Non-smokers: lifetime non-smokers and long-term ex-smokers who stopped smoking ≥1 year before surgery.

Level of significance for comparison of mean adduct levels between genotypes using one way analysis of variance.

Level of significance for comparison of mean adduct levels between smokers and non-smokers (Mann-Whitney U-test).

tissue samples. The estimated DNA adduct levels (adducts/10^8 normal nucleotides) in bronchial tissues of smokers (8.6 ± 4.2, n = 104) were 54% higher (P = 0.0001) than in those of non-smokers (5.6 ± 3.3, n = 39). As shown in Table II, DNA adduct levels in smokers were consistently higher across genotypes of the xenobiotic metabolizing enzymes studied. Therefore, comparison of adduct levels within genotypes was made separately in smokers and non-smokers. Bronchial DNA adduct levels (adducts/10^8 nucleotides) were 16–29% higher in CYP2C9 Ile359 homozygotes than in heterozygotes (8.8 ± 4.3 versus 7.6 ± 3.5 in smokers and 5.8 ± 3.5 versus 4.5 ± 1.3 in non-smokers; Table II and Figure 1). Neither of the differences, however, were statistically significant. There were no CYP2C9 Leu359 homozygotes in this patient group. The CYP2C9 Arg144Cys polymorphism was not associated with DNA adduct levels nor was there a clear relationship between GSTP1 genotypes and DNA adduct levels. Whereas adducts in smoking GSTP1 heterozygotes (Ile105/Val105) and homozygotes (Val105/Val105) were slightly lower than those in homozygotes (Ile105/Ile105), no association was detected in non-smokers. With respect to the NQO1 genetic polymorphism, DNA adduct levels in smokers were not different across homozygous Pro187, heterozygous Pro187Ser and homozygous Ser187 genotypes. The adduct levels in the 18 smokers (10.1 ± 4.6 adducts/10^8 nucleotides) with homozygous frequent alleles of CYP2C9 (Arg144 and Ile359), GSTP1 (Ile105) and NQO1 (Pro187) were slightly higher than that in the smokers who had at least one infrequent allele of the genes studied (8.2 ± 4.0, n = 86).

Discussion

In the present study, the influence of CYP2C9, GSTP1 and NQO1 genotypes on the levels of smoking-related DNA adducts was examined in a Hungarian study population with pulmonary diseases. A significant difference was observed in the bulky DNA adduct levels in bronchial tissues between smokers and non-smokers, as analyzed in more detail previously (28). In this study, the CYP2C9 Ile359 allele was associated with higher adduct levels. CYP2C9 genetic polymorphisms were recently evaluated in relation to lung cancer risk among Caucasians in Los Angeles County, California (25,26). Slightly increased risk of lung cancer was observed with the CYP2C9*2 allele (Cys144, Ile359) (25,26), whereas the CYP2C9*3 allele (Arg144, Leu359) was associated with slightly decreased lung cancer risk (26). Our present results on the CYP2C9 Ile359 allele are consistent with the study in Los Angeles County. On the other hand, no association was found among smoking-related adducts and genotypes of CYP2C9 Arg144Cys, GSTP1 Ile105Val or NQO1 Pro187Ser.

The function of CYP2C9 in the metabolism of tobacco smoke PAH carcinogens such as B[a]P is complex. CYP2C9 has been shown to catalyze both activation and inactivation reactions using cDNA expression systems in bacteria, yeast and mammalian cells (31–33). This bioactivation involves pathways leading to 9-hydroxy-B[a]P-4,5-oxide and also 7,8-diol-9,10-epoxide formation from B[a]P-7,8-diol. In human lung, which is the target tissue of tobacco smoke carcinogens, protein expression of CYP2C9 has been verified along with CYP1A1, CYP2E1, CYP3A4/5 and CYP2D6, although CYP1A1 was the most abundant pulmonary CYP (34,35). In spite of low content of CYP2C9 in lung tissues, a relatively high capacity of metabolic activation of B[a]P by cDNA-expressed CYP2C9 has been reported (31). Furthermore, higher affinity of CYP2C9 for B[a]P was suggested as compared with CYP1A1 and CYP2E1 in a kinetic study on B[a]P metabolism (31). Tissue DNA adducts with BPDE, an ultimate carcinogen formed through the metabolism of B[a]P, were detected in human white blood cells and lungs by HPLC/synchronous fluorescent spectrometry (36,37). Administration of B[a]P-trans-7,8-dihydrodiol and 9-hydroxy-B[a]P to rats resulted in BPDE–DNA adducts and possibly an adduct of 9-hydroxy-B[a]P-4,5-epoxide in lung, liver and peripheral blood (38). This indicates that proximate carcinogens formed through the metabolism of B[a]P were transported to target tissues by circulation and that CYP2C9 is likely to influence the metabolism of tobacco smoke PAHs in lungs as well as in liver, where CYP2C9 is most abundantly expressed.

Highly related, but different alleles which result in amino acid substitutions have been found for CYP2C9 (Arg144Cys and Ile359Leu), GSTP1 (Ile105Val) and NQO1 (Pro187Ser) at a frequency >1% in Caucasians and other populations (18–20). Although six different CYP2C9 sequences have been reported, only two different polymorphic sites in the CYP2C9 gene have been detected in Caucasian and Chinese populations to date (18,30). Little is known, however, on the function of

DNA adduct levels and CYP2C9, GSTP1 and NQO1
Fig. 1. Effect of smoking and different alleles of xenobiotic metabolizing enzymes on bulky adduct levels in bronchial tissue DNA. The levels of bronchial DNA adducts (per 10^8 nucleotides) were compared according to the different alleles of CYP2C9Arg144Cys, CYP2C9Ile359Leu, GSTP1Ile105Val and NQO1Pro187Ser. Genotypes a and b are defined as described in the legend to Table I. Adduct levels in smokers with the frequent allele for each polymorphic site are set as 100%. The 100% values for each genotype are as follows: 8.6 for CYP2C9Arg144 homozygotes; 8.8 for CYP2C9Ile359 homozygotes; 9.2 for GSTP1Ile105 homozygotes; 8.9 for NQO1Pro187 homozygotes. The data are expressed as means ± SD. Numbers in parentheses represent the numbers of subjects. ‘Non-smokers’ includes life-time non-smokers and ex-smokers who stopped smoking more than a year after surgery. ‘Smokers’ includes current smokers and ex-smokers who stopped smoking <1 year before surgery.

the different alleles of CYP2C9 in alteration of enzyme capacities in the bioactivation or detoxification of tobacco smoke carcinogens such as B[a]P, although the amino acid substitutions have been reported to cause impaired (s)-warfarin, tolbutamide and phenytoin metabolism (39–42). The two different forms encoded by GSTP1 showed different catalytic properties depending on the substrate used in cDNA expression systems (43). The amino acid substitution (Pro→Ser) at codon 187 in NQO1 was suggested to result in deficient DT-diaphorase activity toward dichlorophenolindophenol using a human colon cancer cell line (44).

In the present study, levels of bulky DNA adducts were higher in bronchial tissues of those patients who were homozygous for the frequent Ile359 allele of CYP2C9, for which a role in the bioactivation of tobacco smoke carcinogens has been proposed. No clear relationship was observed between adduct levels and the polymorphisms of CYP2C9(Arg144), GSTP1 and NQO1. Although additional studies are needed to verify the function of different alleles of the CYP2C9 gene, our preliminary results and those of others (39–43) indicate that low CYP2C9 activity is associated with increased genotoxicity.

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