Impact of Inherited Polymorphisms in Glutathione S-Transferase M1, Microsomal Epoxide Hydrolase, Cytochrome P450 Enzymes on DNA, and Blood Protein Adducts of Benzo(a)pyrene-diolepoxide

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Abstract

The benzo(a)pyrene (BaP) metabolite benzo(a)pyrene-diolepoxide (BPDE) is strongly implicated as a causative agent of lung cancer. To assess the risk of exposure to BaP, we made a combined analysis of levels of BPDE adducts to hemoglobin (Hb), serum albumin (SA), and lymphocyte DNA in 44 patients with incident lung cancer, as a prototype of a population mainly exposed to tobacco-derived BaP. We also investigated whether genetic polymorphisms of cytochrome P450IA1 (CYP1A1), microsomal epoxide hydrolase (mEH), and glutathione S-transferase M1 (GSTM1), which are involved in BaP metabolism, can be determinants of adduct formation.

BPDE-Hb, BPDE-SA, and BPDE-DNA adducts were quantified as BaP tetrols released from hydrolysis of macromolecules and measured by high-resolution gas chromatography-negative ion chemical ionization-mass spectrometry to achieve high specificity and sensitivity. Individuals with detectable Hb adducts were positive for SA adducts but not vice versa, suggesting that BPDE-Hb adducts are less informative indicators of BaP exposure.

Using PCR methods on DNA, we characterized GSTM1 deletion, CYP1A1 MspI and exon 7 valine variants, and mEH polymorphisms at amino acid positions 113 (EH3) and 139 (EH4). Levels of BPDE adducts were not different among CYP1A1, mEH, and GSTM1 genotypes. However, individuals with measurable BPDE-SA adducts were CYP1A1 variant carriers more frequently (P = 0.03). There was a slightly higher percentage of DNA detectable adducts in subjects with CYP1A1 exon 7 valine polymorphism. When subjects were classified by both polymorphisms on the mEH gene, those with two slow alleles (EH3 homozygous mutated) and no fast alleles (EH4 homozygous wild type) had a lower frequency of BPDE-SA adducts and no DNA adducts (P = 0.06).

These results are based on a small number of observations thus far, but this exploratory study suggests that CYP1A1 and mEH variants might have an impact on BPDE exposure markers such as BPDE-SA adducts. Chemical specificity in adduct measurements is important to identify the biomarkers that reflect BaP exposure more accurately.

Introduction

BaP, a representative of the class of pulmonary carcinogens known as PAHs, is an ubiquitous pollutant, found in automobile exhaust, food, occupational settings, contaminated air, and tobacco smoke. Thus, human exposure to this chemical is widespread and very likely related to overall lung cancer risk (1).

To identify quantitative indicators of human exposure to this carcinogen, work has focused widely on monitoring PAH adducts in relation to BaP exposure and bioactivation in humans. High levels of PAH-DNA adducts in human blood cells and PAH albumin adducts have been associated with high ambient levels of PAH, including BaP (2–4). One study reported a significantly higher level of PAH-DNA in lung cancer cases than in controls, given similar current smoking patterns (5).

Within the p53 tumor suppressor gene, the BaP ultimate carcinogenic metabolite BPDE [(±) r-7, r-8-dihydroxy-r-9, r-10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene] preferentially modifies the guanine residue in the same mutational hot spots as those in human lung cancer (6).

The conversion of BaP into DNA and protein-reactive BPDE is dependent on a cascade of biotransformations, including BaP oxidation by CYP1A1 into benzo(a)pyrene-7,8-oxide, hydration by the mEH into benzo(a)pyrene-7,8-dihydriodiol, and a final P450-dependent oxidation step giving rise to the highly carcinogenic BPDE (7). However, intermediate metabolites and BPDE itself can be detoxified through different pathways like conjugation with glutathione, catalyzed by the GST superfamily, which includes the isoenzyme GSTM1 (7).

Because the genes encoding the enzymes mentioned have been found to be polymorphic in the human population, various studies have tried to relate the metabolic genotype to PAH-
induced cancer risk. The \textit{GSTM1} gene codes for the detoxification enzyme \textit{GSTM1} and is deleted in $\sim 50\%$ of the Caucasian population. The inherited absence of the \textit{GSTM1} gene (the \textit{GSTM1} null genotype) has been associated with a higher risk of lung cancer (8–11).

A mutation in the 3'-noncoding region of the \textit{CYP1A1} gene, creating a new \textit{MspI} cleavage site (\textit{m2}), was overrepresented among lung cancer patients in Japan (12). The existence of a link between this polymorphism and lung cancer is still debated in the Caucasian population, perhaps on account of the considerably lower frequency of the variant allele among Caucasians (13–15).

Conflicting results for a role of \textit{CYP1A1} polymorphism in enhanced susceptibility to PAH carcinogenesis have also been reported for the mutation in the catalytic region ( exon 7) of the gene, closely linked to the \textit{MspI} variant. The A$\rightarrow$G transition creates an allelic variant (\textit{m2}) with an associated amino acid substitution of Val-462 for Ile-462, resulting in enhanced enzymatic activity (16, 17). This polymorphism has been associated with lung cancer in Japanese populations but not clearly in Caucasians, who have a much lower frequency of the mutant trait (14, 18, 19).

Two point mutations have been described in the human \textit{meH} gene, affecting enzyme activity: one, at amino acid position 113 in exon 3, changes tyrosine residue to histidine (His-113), reducing the enzyme activity by at least 50%; another, at amino acid position 139 in exon 4, changes histidine residue to arginine (Arg-139), producing an enzyme with activity that is increased by at least 25% (20).

The low-activity His-113 allele was associated with an increased risk of hepatocellular carcinoma, presumably because of a reduction in the ability to detoxify aflatoxin B1 (21). To date, there are few reports relating mutant alleles of \textit{meH} with particular cancer risk (22, 23), including PAH-induced cancer, although \textit{meH} is an important control point for directing the pathway of BaP detoxification and/or activation.

The question of whether metabolic polymorphisms act as modifiers of exposure biomarkers is currently being widely investigated. The \textit{GSTM1} null genotype has been associated with higher PAH-DNA adduct levels in lung tissue but not in lymphocytes (24, 25). PAH-DNA adduct levels in peripheral WBCs may depend, in part, on particular \textit{GSTM1} and \textit{CYP1A1} genotypes (26, 27), although negative results have been reported as well (24, 28). Thus, the relationship between markers of PAH exposure and markers of metabolic susceptibility is still unclear.

The controversial results might depend partly on differences in the analytical sensitivity and specificity of adduct detection by immunological methods and $^{32}$P-postlabeling assay, in which the exact chemical species responsible is not identified. The lack of chemical specificity in adduct measurements might hinder the identification of any association with specific metabolic polymorphism.

Recently, we quantified BPDE-Hb adducts in a population exposed to low levels of BaP, mainly from traffic exhaust (29), using a gas chromatography-mass spectrometry method that was highly sensitive and specific for BPDE.

In this preliminary investigation, we applied the same method for the measurement of a battery of biomarkers, such as BPDE adducts to Hb, to SA, and to lymphocyte DNA in a group of lung cancer smoker patients as a prototype of a population mainly exposed to tobacco-smoke-derived BaP. We also investigated the relative contribution of inherited metabolic capabilities (\textit{CYP1A1}, \textit{meH}, and \textit{GSTM1}) on adduct profile.

### Materials and Methods

#### Patients and Sampling.
Forty-four male Caucasian patients with incident, histologically confirmed lung cancer were enrolled at the time of their first admission to the European Institute of Oncology in Milan between 1995 and 1997. All were still living their own lifestyle (all current smokers) and had not yet had any pharmacological treatment.

A structured questionnaire was administered by trained interviewers, including information on occupational, dietary, and detailed smoking histories. As an indication of cumulative smoking exposure, pack-years were computed as the average number of packs smoked per day multiplied by years of smoking. The family history of cancer was also recorded. A summary of patients' characteristics, including histological classification, is presented in Table 1.

After giving informed consent, each subject donated 40–50 ml of blood, which was collected in heparinized tubes. Plasma, RBCs, and lymphocytes were separated by the Ficoll-Paque method (Pharmacia Biotech, Cologno Monzese, Italy) within 4 h of sampling. Pelleted cells and plasma were stored at $-80^\circ$C.

#### Isolation of DNA and Proteins.
DNA was extracted from frozen lymphocytes using the procedure described by Wu et al. (30), which requires no organic solvent extraction. Briefly, each lymphocyte pellet was resuspended in 3.5 ml of lysis buffer [400 mM NaCl, 100 mM Tris-HCl (pH 8), 5 mM EDTA, 0.2% SDS, 20 $\mu$g/ml RNase A, and 500 $\mu$g/ml proteinase K] and placed in a 10-cm culture dish at 37$^\circ$C overnight. After digestion, an equal volume of isopropanol was added to each plate and gently mixed until the white DNA precipitate was seen at the bottom of the dish. The dishes were then left undisturbed for 2 h at room temperature. Because the DNA precipitate adheres to the plastic dishes, the supernatant was poured off, and the DNA was washed with ice-cold 70% ethanol. Air-dried DNA was then dissolved in distilled water by incubation at 37$^\circ$C for 2 h.

Albumin was isolated from plasma by addition of saturated ammonium sulfate (final concentration, 50%). After removal of globulins by centrifugation, albumin was precipitated from the supernatant by acidification with 1 M acetic acid (31). Albumin was redissolved in distilled water, and the protein concentration was determined by Bio-Rad protein assay (Bio-Rad, Segrate Milanese, Italy).

Packed RBCs were lysed with 2 volumes of distilled water and centrifuged to remove cell membranes. Hb concentration was measured by Drabkin's method (Drabkin's kit; Sigma, Milan, Italy).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tr>
<td>Total no. of patients</td>
<td>44</td>
</tr>
<tr>
<td>% male patients</td>
<td>100</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
<td>60 (8.3)</td>
</tr>
<tr>
<td>% smokers</td>
<td>100</td>
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<tr>
<td>Cigarettes/day, mean (SD)</td>
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<tr>
<td>Pack-years, mean (SD)</td>
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<td>Squamous cell</td>
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<td>Small cell</td>
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BPDE Adducts Analysis. BPDE adducts to Hb, SA, and DNA were measured essentially as described by Pastorelli et al. (29). Briefly, BPDE adducts were analyzed as BPT released from DNA (200 μg) and proteins (100 mg) after acid hydrolysis of the macromolecules (0.3 N HCl for 60 min at 80°C). DNA hydrolysates did not require any Extrelut purification and were brought to neutral pH and directly loaded onto immunoaffinity columns containing the 8E11 monoclonal antibody developed by Santella et al. (32). BPT were quantified by high-resolution gas chromatography-negative ion chemical ionization-mass spectrometry with selected ion recording, using the stable isotope dilution technique (29).

Determination of Genetic Polymorphisms. All genotypes were determined after gene amplification using PCR. Primers were obtained from Duotech (Milan, Italy). PCRs were performed in the Hybaid Thermocycler (Celbio Srl, Milan, Italy) using Taq DNA polymerase from Promega (M-Medical Sri, Florence, Italy) and dNTP solutions from Epicentre Technologies (Società Prodotti Antibiotici SpA, Milan, Italy).

GSTM1 deficiency was detected as described by Bell et al. (33). β-Globin was coamplified with GSTM1 as internal standard. mEH and CYP1A1 polymorphisms were characterized by RFLP after PCR. All of the restriction enzymes used were obtained from New England Biolabs (Celbio Srl, Milan, Italy). The tyrosine-histidine polymorphism of EH gene at amino acid 113 in exon 3 (EH3) was analyzed by PCR amplification with the primers EPO1 (5'-GATCGATAAGTCCGTT-TCACC) and EPO2 (5'-ATCTTAGCTGTGAAGTGAGGAT), with a base change in primer EPO2 (underlined) to generate an EcoRV restriction enzyme site for samples with the Tyr-113 allele only, according to Smith and Harrison (34).

The histidine-arginine polymorphism at amino acid 139 of mEH gene in exon 4 (EH4) was analyzed by PCR-RFLP using the restriction enzyme Rsal, as described by Hassett et al. (20). The T→C mutation (m1) in the 3'-flanking region of CYP1A1 was detected by PCR-RFLP analysis using the enzymeMsp1 (18). The CYP1A1 Ile/Val replacement (m2) was detected by the improved genotyping method reported by Cascorbi et al. (18), using the BsrDI-RFLP analysis.

Statistical Methods. The correlations between age, tumor histology, smoking habits, and adduct profiles were tested with the Spearman rank-order correlation. To compute mean adduct values, patients with unmeasurable levels were considered as having half the minimum detectable value.

Differences in adduct levels between genotype categories were tested with the Wilcoxon rank-sum test (two categories) and Kruskal-Wallis test (more than two categories).

Fisher’s exact test was used to test the association between genotypes and adduct frequency dichotomized in undetectable and detectable. All tests were computed using SAS software (35).

Results

Individual adduct levels in the 44 patients are presented in Fig. 1, in which Hb and SA adducts are expressed as fmol BPT/mg protein and 1.4 adducts/10⁹ nucleotides.

![Fig. 1. Individual distribution of BPDE-Hb adducts (a), BPDE-SA adducts (b), and BPDE-DNA adducts (c).](image)

The limits of sensitivity were ≤0.05 fmol BPT/mg protein and ≤1.4 adducts/10⁹ nucleotides.

Fig. 1. Individual distribution of BPDE-Hb adducts (a), BPDE-SA adducts (b), and BPDE-DNA adducts (c). Each sample was run in duplicate. Data points, average values. Samples with undetectable adducts are grouped (bottom). The limits of sensitivity were ≤0.05 fmol BPT/mg protein and ≤1.4 adducts/10⁹ nucleotides.

Briefly, BPDE adducts were analyzed as BPT released from DNA (200 μg) and proteins (100 mg) after acid hydrolysis of the macromolecules (0.3 N HCl for 60 min at 80°C). DNA hydrolysates did not require any Extrelut purification and were brought to neutral pH and directly loaded onto immunoaffinity columns containing the 8E11 monoclonal antibody developed by Santella et al. (32). BPT were quantified by high-resolution gas chromatography-negative ion chemical ionization-mass spectrometry with selected ion recording, using the stable isotope dilution technique (29).

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Results

Individual adduct levels in the 44 patients are presented in Fig. 1, in which Hb and SA adducts are expressed as fmol BPT/mg protein hydrolyzed (Fig. 1, a and b, respectively). DNA adducts are shown as number of adducts/10⁹ nucleotides (Fig. 1c).

The limit of sensitivity for the detection of BPT was ≤0.05 fmol/mg in human proteins, and in DNA, it was ≤1.4 adducts/10⁹ nucleotides.

BPDE-HB adducts were detectable in six subjects (13.6%) with an overall median level of 0.025 fmol BPT/mg Hb. BPDE-SA adducts were measurable in 24 patients (56.8%; median, 0.11 fmol BPT/mg SA). BPDE-DNA adducts were detectable in 18 (38.6%; median 0.7 adducts/10⁹ nucleotides).

When adduct levels were classified as detectable or undetectable, the presence of Hb adducts was significantly associated with the presence of SA adducts (P = 0.029; Fisher’s exact test). No associations were found with BPDE adducts to DNA.

There was a correlation between number of cigarettes smoked daily and DNA adducts (r = 0.34, P = 0.02). Cumulative smoking, expressed as pack-years, was associated with the DNA adduct level (r = 0.3, P = 0.05). No further associations were found.

The distribution of the genotypes is shown in Fig. 2. Twenty-three patients (52.3%) were GSTM1 null genotype, which results from the total absence of the gene.

Absence of the Msp1 site in both alleles, representing the homozygous wild-type genotype (wt2/wt1), was seen in 38 patients (86.4%), and the remaining 6 (13.6%) were heterozygous (wt1/m1). Point mutational Ile-Val polymorphism (m2) in the exon 7 of CYP1A1 gene was found as a heterozygous conformation (wt2/m2) in three patients (6.8%), whereas the
rest were homozygous wild-type genotype (wt2/wt2). The presence of one copy of the exon 7 variant polymorphism (m2) was significantly associated with the MspI polymorphism (m1; \( P = 0.001 \), Fisher’s exact test, two-tailed). Because mutated alleles \( m1 \) and \( m2 \) are rare in the Caucasian population, no homozygous carriers were found in our group.

The EH3 polymorphism on \( mEH \) gene was detected in 50% of the population studied, with 25% being homozygous for the mutated allele and 25% being heterozygous. Twenty-two were genotyped as homozygous wild type (50%).

The majority of the subjects (61.4%) were classified as homozygous wild type for the EH4 polymorphism on \( mEH \) gene. There were 16 heterozygous carriers (36.3%), and only 1 (2.3%) had the homozygous mutated allele.

Table 2 shows the mean levels of BPDE adducts according to each genotype. The GSTM1 genotype did not have any effect on BPDE-SA and DNA adducts. BPDE-Hb adducts were higher in the GSTM1 null group than in the GSTM1 positive genotype, but the difference was not significant. Slightly higher levels of Hb adducts were also observed in the homozygous wild type for each CYP1A1 variant (wt1/wt1 and wt2/wt2) compared to the heterozygous conformation (wt1/wt2 and wt2/wt2). Increased levels of SA and DNA adducts found in the presence of each variant were not significantly associated with the heterozygous genotypes.

No significant influence on Hb, SA, and DNA adducts emerged with the presence of at least one mutated allele for EH3 and EH4 polymorphisms.

The presence of adducts was then divided into positive and undetectable. The frequency of detectable adducts in relation to each genotype is reported in Fig. 3. Individuals with measurable Hb and DNA adducts were more frequently GSTM1 null genotype (Fig. 3A), but this tendency was not significant.

We classified the patients by CYP1A1 polymorphisms in three groups because the three who had the rare \( m2 \) polymorphism were a subset of those with the rare \( m1 \) variant (Fig. 3B). When detectable adducts were analyzed in relation to these genotypes, the percentage of positive SA adducts was higher among the heterozygous carriers than the wild type. A significant association \( (P = 0.03) \) was only found in SA adducts when subjects with at least one variant were grouped together and compared to those with no mutation on the gene. A non-significant doubling of detectable DNA adducts (66%) was observed in the presence of the \( m2 \) variant compared to the wild-type (36.8%) and \( m1 \) mutated allele (33%) conformations.

Patients homozygous and heterozygous for EH3 variant tended to have fewer detectable adducts to SA and DNA than those who were wild type (Fig. 3C), but this difference was not significant. The EH4 genotype was not associated with adduct frequency (Fig. 3D).

Interestingly, after classification of subjects by both \( mEH \) gene polymorphisms (EH3 and EH4), the six who had two mutated alleles on EH3 and no mutation on EH4 showed no detectable DNA adducts and a lower frequency of detectable SA adducts than those presenting the remaining haplotype combinations, as shown in Fig. 4 \( (P = 0.06 \) and \( P = 0.3) \).

**Discussion**

For this preliminary investigation, we selected a group of lung cancer patients who had a long history of smoking and were...
Fig. 3. Frequency of detectable adducts in relation to GSTM1 genotype (A), CYP1A1 genotype (B), EH3 genotype (C), and EH4 genotype (D). GSTM1 +, homozygous positive and heterozygous genotypes; GSTM1 −, homozygous null genotype; CYP1A1 m1, MspI mutated allele; CYP1A1 m2, exon 7 mutated allele; EH3 m1, mEH exon 4 mutated allele. Numbers in brackets, total numbers of subjects carrying each genotype; numbers in parentheses, numbers of subjects with detectable adducts. * P = 0.03, Fisher’s exact test, two-tailed, no mutation versus at least one mutated allele. None of the other differences were significant.

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still smokers at the time of blood sampling, thus representing a prototype of subjects heavily exposed to the tobacco carcinogen BaP. Neither occupational nor dietary BaP exposure was elicited by the questionnaire.

A unique aspect of our approach is the use of a highly specific method based on BPT released from hydrolysis of the macromolecules and measured by high-resolution gas chromatography-negative ion chemical ionization-mass spectrometry, which achieves high sensitivity with a detection limit of 1.4 adducts/10^9 nucleotides and 0.05 fmol BPT/mg protein.

This is the first comparative analysis of multiple dosimeters such as BPDE adducts to Hb, SA, and DNA in the same population.

Our method found lower levels of BPDE adducts to proteins and DNA than those measured by other authors in smokers (27, 36–38). This difference may be due to the fact that the immunoassay techniques used by these authors measured several types of PAH adducts due to cross-reactivity of the antibody used.

The BPDE-Hb adducts levels we found were lower, but comparable to those recently reported in smokers using a gas chromatography-mass spectrometry approach (39).

The percentage of individuals with detectable BPDE-Hb adducts was lower than that of individuals with measurable SA adducts. Individuals presenting Hb adducts were positive for SA adducts but not vice versa, suggesting that BPDE-Hb adducts might be less informative than expected as a marker of internal dose.

Several studies report that PAH-DNA adducts in leukocytes are associated with cigarette smoking and decline after stopping smoking, (40) but the structure of the adducts has never been reported. Here, we have shown that a significant association exist between BPDE-DNA adduct levels in lymphocytes and cigarettes/day as well as pack-years.

The lack of correlation between blood proteins and DNA adducts in the same subject might be due to differences in kinetics of these biomarkers (41).

The level of BPDE adducts is expected to be affected not only by the exposure dose of BaP but also by metabolic polymorphism of the enzymes involved in its metabolism.

In our study, the GSTM1 genotype did not have any significant effect on BPDE adducts formation, possibly due to the small sample size, but individuals with detectable Hb and DNA adducts were more frequently GSTM1 null genotype.

Conflicting results are reported on how the polymorphic CYP1A1, the key enzyme in activation of many carcinogens, affects lymphocyte DNA adducts.

Shields et al. (24) showed no correlation between PAH-DNA adduct levels and CYP1A1 m1 variant in lung cancer patients. Similarly, Hemminki et al. (42) observed only a bor-
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Fig. 4. Frequency of detectable adducts in relation to mEH gene polymorphisms. EH3 m/m and EH4 w/w, homozygous mutated on exon 3 and homozygous wild type on exon 4, respectively. Numbers in brackets, total numbers of subjects carrying each genotype; numbers in parentheses, numbers of subjects with detectable adducts. None of the differences were statistically significant.

derline{effect} in foundry workers. Mooney et al. (36) reported that heavy smokers with CYP1A1 m2 variant alone or in combination with GSTM1 null genotype sustained more genetic damage from cigarette smoking than individuals without these factors.

In our study, subjects with undetectable adducts did not carry any CYP1A1 polymorphism; the presence of at least one variant in the CYP1A1 gene was significantly associated with higher frequency of detectable BPDE-SA adducts but not with Hb adducts or with DNA adducts. To our knowledge, this is the first evidence that the CYP1A1 polymorphism influences adducts other than DNA adducts.

CYP1A1 polymorphism is often explained as enhanced inducibility, leading to higher enzymatic activity to activate precarcinogens. It has been proposed that m1 mutation in the 3' flanking region of the gene might be a marker for alteration on regulatory sites, whereas m2 seems to increase enzymatic activity (16, 17). In both cases, the activation of BaP to the ultimate carcinogenic BPDE might be enhanced, resulting in higher levels of SA adducts. This preferential site of adduction could be easily explained by the fact that adduct formation may be more efficient with SA because the protein is synthesized in the liver, in which most xenobiotic metabolism takes place. But CYP1A1 is considered to be primarily an extrahepatic enzyme, so its apparently enhanced activity would not sustain the increased formation of BPDE adducts to SA. This apparent discrepancy may be due to the influence of CYP1A1 polymorphisms on the function of CYP1A2, a cytochrome P450 enzyme responsible for BaP activation in the liver. It has been recently reported that on exposure to cigarette smoke, individuals with heterozygous variants of CYP1A1 gene had significantly increased CYP1A2 activity (43).

In our study, the small number of people with the rare m2 allele precluded any analysis stratified by exon 7 genotype, reported to be the “high-risk” polymorphism for lung cancer (17). There was only a suggestion that the m2 variant influenced BPDE-DNA adduct frequency.

We focused our attention on a further enzyme involved in the BaP metabolism: mEH, the polymorphism of which has not been evaluated in relation to PAH exposure.

The mutation on exon 3 of the mEH gene (EH3) that changes Tyr-113 to His-113 reduced the enzyme activity, yielding a slow allele, whereas the mutation on exon 4 (EH4) changing His-139 to Arg-139 and increased the activity, yielding a fast allele (20).

Considering each variant separately, no remarkable effects on adduct levels or frequency were noted. If the patients are classified by both polymorphisms, those with two mutated alleles on exon 3 and no mutation on exon 4 had a lower frequency of BPDE-SA adducts and a marginally significant absence of DNA adducts.

The mechanism underlying this finding can be explained on the basis of mEH enzymatic activity as a function of polymorphic 113 and 139 amino acid residues.

Patients with two slow alleles (EH3 homozygous mutated) and no fast allele (EH4 homozygous wild type) are a very slow phenotype for which decreased enzyme activity can be inferred. In the case of BPDE adduct formation, this mEH genotype might constitute a protective genetic make-up.

However, the role of mEH polymorphisms in relation to cigarette smoke carcinogens has to be evaluated carefully because the mEH slow phenotype has been correlated with increased susceptibility to emphysema, suggesting that the putative decrease in enzyme activity is responsible of the increased epoxide intermediates involved in the progressive tissue abnormalities seen in emphysema (34).

The correlation between mEH polymorphism and enzyme activity needs to be clarified, and a recent study was unable to assign hepatic mEH protein/enzymatic activity levels solely based on the two polymorphic amino acid loci, suggesting that additional factors regulate mEH expression (44).

In conclusion, our findings address the importance of comparative biomarker analysis to identify the exposure marker that most accurately indicates the true dose absorbed. This explorative investigation shows that BPDE-SA adducts are apparently more easily influenced by genetically determined differences in metabolizing BaP than are Hb and lymphocyte DNA adducts.

Although our patients were a homogeneous population with respect to tobacco BaP exposure, at least in terms of cigarettes/day and pack-years, the effect of genotypes could not completely explain the high variability in adduct levels observed, suggesting others factors, such as type of tobacco or smoking style, might affect the dose of BaP received.

This study brought to light some suggestions of a relation between the genotypes considered and BPDE adduct formation, but the small sample size prevented any conclusive inference.

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References


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