Environmental Health Perspectives
Vol. 98, pp. 191–194, 1992

Polycyclic Aromatic Hydrocarbon–DNA Adducts and the CYP1A1 Restriction Fragment Length Polymorphism

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Human cancer risk assessment at a genetic level involves the investigation of carcinogen metabolism and DNA adduct formation. Wide interindividual differences in metabolism result in different DNA adduct levels. For this and other reasons, many laboratories have considered DNA adducts to be a measure of the biologically effective dose of a carcinogen. Techniques for studying DNA adducts using chemically specific assays are becoming available. A modification of the ^{32}P-postlabeling assay for polycyclic aromatic hydrocarbon DNA adducts described here provides potential improvements in quantification. DNA adducts, however, reflect only recent exposure to carcinogens; in contrast, genetic testing for metabolic capacity indicates the extent to which carcinogens can be activated and esert genotoxic effects. Such studies may reflect both separate and integrated risk factors together with DNA adduct levels. A recently described restriction fragment length polymorphism for the CYP1A1, which codes for the cytochrome P450 enzyme primarily responsible for the metabolic activation of carcinogenic polycyclic aromatic hydrocarbons, has been found to be associated with lung cancer risk in a Japanese population. In a subset of individuals enrolled in a U.S. lung cancer case-control study, no association with lung cancer was found.

Introduction

Carcinogenesis is a multistage process involving initiation, promotion, conversion, and progression. Carcinogens can influence these stages through the formation of DNA adducts. The level of carcinogen DNA adducts is the end result of exogenous exposure, internal absorption, metabolic activation, detoxication, and DNA repair; however, substantial interindividual variations in these activities have been observed. Techniques for studying both carcinogen metabolism (phenotyping and genotyping) and carcinogen exposure (carcinogen-macromolecular adducts) are now becoming available. These can be viewed as separate or integrated cancer risk factors. Measurement of carcinogen-DNA adducts (or a surrogate) reflects recent exposure (days to months), while metabolic phenotyping or genotyping reflects a capability possessed throughout the lifetime of an individual.

Polycyclic aromatic hydrocarbons (PAHs), metabolized by cytochrome P450 1A1 (CYP1A1) and other cytochrome P450s, are ubiquitous in the environment and can cause cancer in laboratory animals. Adducts can be promutagenic, display site specificity, and activate proto-oncogenes (*1–3*). Adduct levels can be measured in humans by enzyme immunoassays and have been found to be correlated with exposure to coke-oven emissions, foundry work, tobacco, and diet (*4–6*) although not consistently. Putative adducts have also been measured by the ^{32}P-postlabeling asha) (*7*), and levels have been correlated with tobacco consumption (*8,9*); however, the lack of chemical specificity of the current assay limits its ability to determine the actual level and adduct type.

CYP1A1 is partly responsible for the metabolic activation of PAHs. It is inducible by exposure to these compounds and other agents, e.g., 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. No direct measure of the capacity of humans to metabolize PAHs has been developed, although the results of cell and tissue culture studies indicate that wide interin-

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dividual variation exists. Moreover, higher levels of aryl hydrocarbon hydroxylase activity and PAH adduct formation have been reported in lymphocytes of cancer patients compared to controls (*10,11*). Levels of wild-type CYP1A1 mRNA have been measured in normal lung tissue, but no correlation with lung cancer risk was found although an abnormal mRNA species was identified in tumor tissues (*12*). Significantly higher levels were found in tobacco smokers, confirming the inducibility by this agent. A recent report by Kawajiri et al. (*13*) stated that an *MspI* restriction fragment length polymorphism (RFLP) was positively correlated with lung cancer risk in a Japanese population. Individuals homozygous for the *MspI* restriction site-present, allele had an odds ratio for cancer of 3.1 when compared to individuals homozygous for the site-absent allele (predominant allele). The association was strongest in people with squamous-cell carcinoma of the lung. An important limitation of this study, however, was use of data for historical control subjects; specifically, the study was not controlled for either age or tobacco use. We have now performed a pilot epidemiologic study of 78 individuals, controlling for age, race, and smoking.

Methods

Analysis of CYP1A1 Polymorphism

DNA samples from 78 individuals enrolled in a case-control study of lung cancer were analyzed using the polymerase chain reaction (PCR) and *MspI* restriction digest. Primers flanking a polymorphic *MspI* restriction site were used to amplify a 348-bp fragment of genomic DNA (*13*). Products of PCR were then digested to completion with *MspI* and analyzed on agarose gels (1.8%).

^{32}P-Postlabeling Assay

Benzo[*a*]pyrene–DNA adduct standards were synthesized by modifying calf thymus DNA with *r*-7,*t*-8-dihydroxy-t-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (benzo[*a*]pyrene diol epoxide). Modified DNA was enzymatically digested to 3′-monophosphate-nucleotides and purified by reverse-phase high-performance liquid chromatography. Adduct levels were quantified on the basis of ultraviolet extinction coefficients and fluorescence spec-

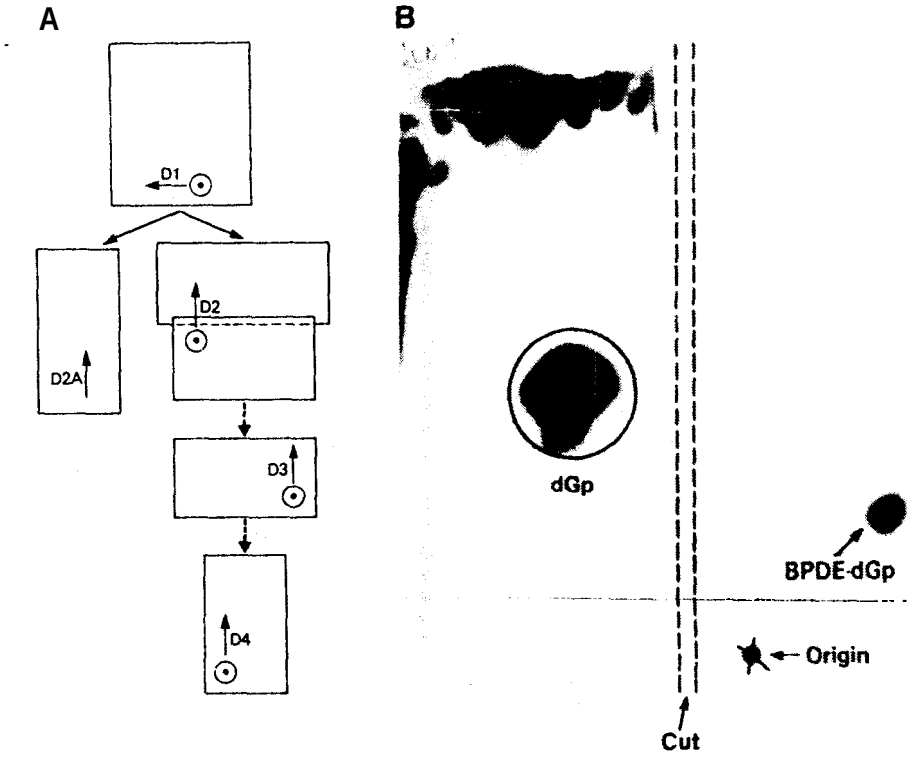


FIGURE 1. Chromatographic separation (*A*) allows for localization and quantification of unmodified 2′-deoxyguanosine-3′-monophosphate (dGp) and benzo[*a*]pyrene-diol epoxide (BPDE)-dGp. After development in D1 (1.25 LiCl), the plates are washed in methanol and then cut in half with scissors. Each plate is then developed separately. The left-hand side with the unmodified nucleotide, is developed in 2A (saturated ammonium sulfate/isopropanol/1 M sodium acetate: 80/2/18 v/v) while the right-hand side is developed in D2B (2.3 M sodium phosphate, pH 6), D3 (3.5 M lithium formate/8.5 M urea, pH 3.5), and D4 (1.2 M lithium chloride/0.5 M Tris-HCl/8.5 M urea, pH 8.0). A typical autoradiograph is shown in which the right and left halves have been rejoined for demonstration purposes. The cutting area is shown.

troscopy. The adducts were then mixed, in known quantities, with 70 pmole of 2'-deoxyguanosine-3'-monophosphate (dGp) (internal standard) and postlabeled as previously reported (14) except that kinase treatment was for 30 min. The samples were then applied to polyethyleneimine cellulose thin-layer chromatography plates (Machery Nagel, Germany; 20 × 20 cm) and developed in multiple dimensions, as described (Fig. 1). Unmodified dGp and adducts were localized by autoradiography and scraped, and the radioactivity was measured by liquid scintillation counting. Direct molar ratios of adduct level to dGp were then determined on the basis of counts per minute.

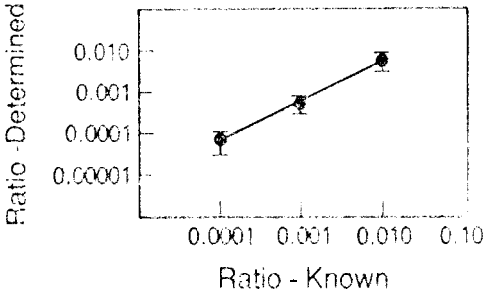


FIGURE 3. Calibration curve showing directly determined molar ratios for benzo[*a*]pyrene-diol epoxide-2'-deoxyguanosine-3'-monophosphate/2'-deoxyguanosine versus the known ratio.

Results and Discussion

The recently described *Msp*I polymorphism, which correlates with lung cancer risk in Japanese individuals, was tested in random individuals and in 78 U.S. study subjects accrued in a formal case–control study of lung cancer. A comparison was made between Southern blot analysis using CYP1A1 cDNA (kindly provided by F. J. Gonzalez, National Cancer Institute, Bethesda, MD) and PCR analysis (Fig. 2). There was 100% agreement between the two methods, except for one individual who had a single band at 1.3 kb (rather than 2.3 and or 1.9 kb). The PCR analysis showed that this person had a homozygote site-absent genotype indicating deletion of a segment 5' to the MspI polymorphic site. Further analysis of this individual is warranted.

The genotype distributions in the case-control study (homozygote site-absent, heterozygotes, and homozygote site-present) were not significantly different in cases and controls. A trend indicating more site-present alleles in people with squamous-cell carcinoma than in those with other histological types was found, but this was not statistically significant. These data suggest that in a U.S. population, the *Msp*I polymorphism of CYP1A1 is not associated with an increased risk for lung cancer. We are currently expanding this study by analyzing additional samples to generate sufficient power to detect a shift in allelic frequencies in racial subgroups.

The biological relationship of the *Msp*I polymorphism, which is located approximately 1.5 kb to the 3' end of the *CYP1A1* structural gene, remains undetermined. Studies are under way to determine if there is an association with aryl hydrocarbon hydroxylase induction and activity or with CYP1A1 mRNA levels. Should an association of this polymorphism with biological function exist *in vivo*, then an association with PAH–DNA adduct levels can also be sought.

Determination of carcinogen adduct levels by the ^{32}P-postlabeling assay is useful for testing the biological significance of interindividual variation in metabolism; such adducts are probably also an important independent risk factor for cancer. This assay has been widely used for the detection of adducts in single and multiple biological systems; however, inherent limitations result from difficulties in determining specific PAH adduct levels.

We are attempting to develop a chemically specific assay to complement the use of the ^{32}P-postlabeling assay in



FIGURE 2. DNA samples were amplified by polymerase chain reaction and then digested with *Msp*I. A reverse image of an agarose gel (1.5%) shows homozygous site-absent (lanes C, D, F, H, I, J), heterozygous (lanes A, B, E), and homozygous site-present (lanes G, K, L) allelotypes.

screening. A similar strategy to that used for detection of alkyl adducts has been employed (1.5). To this end, we are modifying the ^{32}P-postlabeling assay to include the use of an internal standard (dGp); incorporating high performance liquid chromatography for adduct purification and assessing enzymatic digestion: and developing calibration curves using authentically synthesized DNA adducts and determining molar ratios, rather than relying on determinations of relative adduct labeling that assume complete labeling efficiency. A representative autoradiogram for 7*R*,8*S*,9*S*-trihydroxy-10*R*-(*N*^{2}-deoxyguanosyl-3′-phosphate)-7,8,9,10-tetrahydrobenzo[*a*]pyrene is shown in Figure 1. The labeling efficiency was found to vary slightly by adduct concentration (Fig. 3), consistent with the findings of other authors (16,17). The resulting limit of detection was 7.0 fmole of adduct.

This manuscript was presented as a poster at the Conference on Biomonitoring and Susceptibility Markers in Human Cancer: Applications in Molecular Epidemiology and Risk Assessment that was held in Kailua-Kona, Hawaii, 26 October–1 November 1991.

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