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Lung DNA Adducts Detected in Human Smokers Are Unrelated to Typical Polyaromatic Carcinogens

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Several studies have reported the presence of DNA adducts derived from benzo(*a*)pyrene and other polyaromatics by ^{32}P-postlabeling/TLC by measuring diagonal radioactive zones (DRZs) in lung tissues of human smokers. However, our experimental studies in rodent models, which used modified chromatographic conditions to obtain distinct adduct spots, suggested that cigarette smoke-related lipophilic DNA adducts may not be derived from polycyclic aromatic hydrocarbons (PAHs) or aromatic amines. In the present study, we have performed similar analysis of human lung tissues to study the chemical nature of DNA adducts. Fifty human lung tissues from cancer patients (ages 42−83 years) with active, ex-, or never-smoking status were analyzed for highly lipophilic DNA adducts by nuclease P1- and *n*-butanol enrichment-mediated ^{32}P-postlabeling assay. All DNA samples yielded low to highly intense adduct DRZs when adducts were resolved by PEI-cellulose TLC in standard high-salt, high-urea solvents. Adduct burden ranged from 6.6 to 2930 per 10^{10} nucleotides. However, when adducts were resolved in a different solvent system comprising of high-salt, high-urea in direction 3 and dilute ammonium hydroxide in direction 4, which retained adducts derived from PAHs and aromatic amines on the chromatograms, this yielded no detectable adducts from human lung DNAs. Furthermore, analysis of human lung DNAs mixed with reference adducted DNAs in multisolvent systems confirmed an absence of PAH- and aromatic amine-derived adducts in human smoker lung DNA. To determine the origin of cigarette smoke-associated DNA adducts, calf thymus DNA was incubated with formaldehyde and acetaldehyde, which are known to be present in cigarette smoke in significant quantities. Analysis of purified DNAs by ^{32}P-postlabeling resulted in adduct DRZs in the aldehyde-modified DNAs when adducts were resolved in standard urea-containing solvents, but no adducts were detected when the ammonium hydroxide-based solvent was used, suggesting that even nonpolyaromatic electrophiles can result in adduct DRZs on the chromatograms similar to those from PAH metabolites. Taken together, our data demonstrate that cigarette smoke-associated lung DNA adducts appear on chromatograms as DRZs, consistent with the literature, but they are not related to PAHs and aromatic amines.

Introduction

Lung cancer is the leading cause of cancer-related deaths in the United States, and the lung cancer deaths in the new millennium are estimated at 25% for females and 32% for males of the total cancer mortality (*1*, *2*). Epidemiological studies strongly implicate cigarette smoking as a major etiological factor in the incidence of lung cancer (*3*, *4*). Despite improvement in smoking cessation programs and antitobacco smoking awareness, about 25% of the U.S. adult population continues to smoke cigarettes, a percentage which has remained largely constant since 1990 (*2*). Epidemiological studies have also indicated possible gender-specific susceptibility to lung cancer (*5*, *6*). In contrast to men, the incidence of lung cancer in women over the past decade has increased despite lower overall consumption of cigarettes by women (*2*, *6*).

Cigarette smoke is a complex mixture of chemicals and contains more than 50 known or probable human carcinogens, including polycyclic aromatic hydrocarbons (PAHs),[1] aromatic amines (AAs), and tobacco-specific nitrosamines (*3*, *7*). Many

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[1] Abbreviations: AAs, aromatic amines; 4-ABP, 4-aminobiphenyl; ABZ, N′-acetylbenzidine; AF, 2-aminofluorene; *anti*-BADE, benz[*a*]anthracene-3,4-dihydrodiol-1,2-epoxide (*anti*); *anti*-BFDE, benzo[*k*]fluoranthene-8,9-dihydrodiol-10,11-epoxide (*anti*); BP, benzo(*a*)pyrene; *anti*-BPDE, benzo-(*a*)pyrene-7,8-dihydrodiol-9,10-epoxide (*anti*); *anti*-DBADE, dibenz(*a*,*h*)-anthracene-1,2-dihydrodiol-3,4-epoxide (*anti*); DRZs, diagonal radioactive zones; ETS, environmental tobacco smoke; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; PAH, polycyclic aromatic hydrocarbons; PEI, polyethyleneimine; RAL, relative adduct labeling; TLC, thin-layer chromatography.

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of these compounds possess mutagenic activity, cause DNA damage, and form DNA adducts (*8*). Covalent interaction of DNA with the electrophilic metabolites of carcinogens is thought to be an essential early step in the initiation of cancer (*8*). Despite the diversity of chemical carcinogens in tobacco smoke, an analysis by ^{32}P-postlabeling, fluorescence and immunoassays, and so forth has suggested the presence of PAH- and AA-derived DNA adducts in various human tissues of smokers (*7*, *9*−*12*). However, our experimental studies have failed to detect benzo(*a*)pyrene (BP)- and 4-aminobiphenyl (4-ABP)-derived DNA adducts in the respiratory (lung, trachea) and nonrespiratory (bladder, heart) tissues of smoke-exposed rodents (*13*−*15*). Analysis of environmental tobacco smoke (ETS)-induced lung tumor multiplicity in the A/J mice have indicated that the vapor phase of ETS is as tumorigenic as whole ETS, suggesting that the tobacco-specific nitrosamines such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) or BP may not be involved in tumor induction in that particular A/J mouse model (*16*).

Improvements in the resolution of DNA adducts produced by various carcinogens, including PAHs, nitrated PAHs, and AAs, using an ammonium hydroxide-based chromatographic solvent system (*17*), has prompted us to re-examine lung tissues from human smokers for the presence of PAH- and AA-derived DNA adducts. We postulate that DNA adducts produced in the tissues of human smokers, as diagonal radioactive zones (DRZs) in standard urea-based solvent systems, may not represent the typical PAH- and AA-derived DNA adducts. In the present study, absence or presence of BP- and 4-ABP-derived DNA adducts were analyzed in lung tissues from human lung cancer patients.

Materials and Methods

Subjects and Sampling. Lung tumor tissue samples were obtained from 50 (26 male and 24 female) lung cancer patients (42−83 years of age), with current ($n = 25$), ex- ($n = 22$), or never-smoking ($n = 3$) status, who were undergoing surgical resection of their tumors at Fox Chase Cancer Center, Philadelphia, PA. The ex-smokers included in the study were those who had quit smoking for at least 1 year prior to the surgery. The Institutional Review Boards of Fox Chase Cancer Center and the University of Kentucky approved the protocols. A detailed questionnaire regarding the smoking history (e.g., age of initiation, age at the time of quitting, and pack-year history, etc.) was administered at the time of their scheduled visits to the thoracic surgery clinic. The tissues were collected and stored at −80 °C until shipment on dry ice to the University of Kentucky for analysis.

Preparation of Aldehyde-Derived DNA Adducts. Calf thymus DNA (300 μg) was incubated with formaldehyde and acetaldehyde (40 mM) for 3 h at 37 °C, followed by DNA precipitation with sodium chloride and ethanol (*18*).

Reference DNA Adducts. Calf thymus DNAs adducted with *anti*-diolepoxides of dibenz(*a*,*h*)anthracene (dibenz(*a*,*h*)anthracene-1,2-dihydrodiol-3,4-epoxide; *anti*-DBADE), benz(*a*)anthracene (benz[*a*]anthracene-3,4-dihydrodiol-1,2-epoxide; *anti*-BADE), benzo(*k*)-fluoranthene (benzo[*k*]fluoranthene-8,9-dihydrodiol-10,11-epoxide; *anti*-BFDE), and benzo(*a*)pyrene (benzo(*a*)pyrene-7,8-dihydrodiol-9,10-epoxide; *anti*-BPDE) were kindly provided by Drs. C. C. Harris and A. Weston, National Cancer Institute, Bethesda, MD, and DNAs adducted with *N*-hydroxy derivatives of *N*′-acetylbenzidine (ABZ), 4-aminobiphenyl (ABP) and 2-aminofluorene (AF) were obtained from Drs. F. F. Kadlubar and F. A. Beland, National Center for Toxicological Research, AR. These reference DNA adducts have been characterized in ^{32}P-postlabeled form previously (*19*). The stock adducted DNAs (containing 1 adduct per 10^4−10^6 nucleotides) were diluted with untreated calf thymus DNA to a desired level of approximately 1 adduct per 10^7 nucleotides prior to their use in this study. Two reference adduct mixtures were prepared, one containing PAH-related adducts and the other containing aromatic amines, with each adduct present at approximately 1 per 10^8 nucleotides to 5 per 10^{10} nucleotides. Adduct levels were determined by ^{32}P-postlabeling as described (*19*).

DNA Isolation. DNA was isolated by a phenol extraction procedure as described elsewhere (*18*). Briefly, lung tissues were homogenized in 50 mM Tris-HCl (pH 8.0) containing 10 mM EDTA and 5 mM butylated hydroxy toluene as a free radical inhibitor. Isolated crude nuclei were digested with ribonucleases A (150 μg/mL) and T1 (1 unit/μL) (Sigma Chemical Co., St. Louis, MO), followed by digestion with proteinase K (150 μg/mL) (Boehringer Mannheim Corp., Indianapolis, IN). The samples were extracted sequentially with phenol, phenol/Sevag (chloroform/isoamyl alcohol, 24:1), and Sevag, and DNA was precipitated with 1 vol of ethanol and 0.5 M sodium chloride. The DNA concentration was estimated spectrophotometrically using 1 A_{260} unit as equal to 50 μg of DNA.

DNA Adduct Analysis. DNA (10 μg) was digested with a mixture of micrococcal nuclease (Sigma Chemical Co., St. Louis, MO) and spleen phosphodiesterase (Boehringer Mannheim Corp., Indianapolis, IN) (enzyme/DNA = 1:5, w/w, 5 h at 37 °C). For human lung DNA and PAH- and aldehyde-adducted DNAs, adducts were enriched by treatment with nuclease P1 (Calbiochem-Novabiochem Corp., San Diego, CA) (enzyme/DNA = 1:2.5, w/w, 45 min at 37 °C) (*18*). For selected human lung DNA and AA-adducted DNA, adducts were also enriched by butanol extraction (*18*). Adducts were labeled with molar excess of [γ-^{32}P]ATP, prepared enzymatically using ^{32}Pi (ICN Pharmaceutical, Inc., Costa Mesa, CA) (*18*), and resolved by multidirectional polyethyleneimine (PEI)-cellulose TLC in the following solvent systems. (i) System I: D1 = 1 M sodium phosphate, pH 5.7; D3 = 4 M lithium formate/8.5 M urea, pH 3.5; D4 = 0.8 M lithium formate/8.5 M urea/0.5 M Tris-HCl, pH 8.0; and D5 = 1 M sodium phosphate, pH 5.7. (ii) System II: All solvents were the same as in system I, except that D4 solvent was substituted with 2-propanol/4 M NH_4OH (1.2:1) 2 cm onto Whatman No. 1 paper wick. An aliquot of diluted DNA digest (2 ng) was also labeled in parallel with adducts, and normal nucleotides were resolved in 1.5 M ammonium formate, pH 3.5. Adduct DRZs were visualized and quantified by Packard InstantImager (Packard Instrument Co., Inc., Downers Grove, IL). For quantification, the background radioactivity (cpm/mm^2) from the blank area of the chromatogram was subtracted from the radioactivity of adduct DRZ. The relative adduct labeling (RAL) was calculated as follows: RAL = (cpm in adducts/cpm in total nucleotides) × (1/dilution factor).

Co-Chromatography. Selected human lung DNA specimens were analyzed alone or after mixing with reference PAH- and AA-adducted DNAs, ranging from 5 adducts/10^{10} to 1 adduct/10^8 nucleotides. DNA digestion, enrichment, and labeling conditions were the same as described above. The labeled digests were chromatographed in both solvent systems I and II.

Statistical Analysis. The transformed DNA adduct levels (log[adduct level]) per 10^{10} nucleotides were analyzed by means of Generalized Regression models, and the computations were carried out with SAS statistical software (SAS version 8). $P < 0.05$ was considered significant.

Results

All samples of human lung DNA produced typical adduct maps showing DRZs when analyzed by the nuclease P1-version of ^{32}P-postlabeling and chromatographed in standard urea-based solvent system I (Figure 1A,B). In general, more intense adduct DRZs were observed in lung DNA from smokers (Figure 1A,B) than in the ex- or never-smokers (not shown). However, D4 development in dilute ammonium hydroxide-based solvent system II failed to yield any adduct spots even after a prolonged autoradiographic exposure (Figure 1D,E). Use of butanol extraction-mediated adduct enrichment resulted in diffused

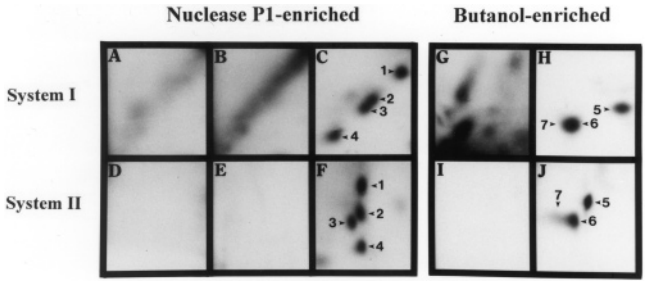

Figure 1. Representative ^{32}P-adduct maps from human smoker lung DNA and reference adducts of selected polycyclic aromatic hydrocarbons (PAHs) and aromatic amines. Maps A−F were derived after enrichment of the adducts by nuclease P1, while maps G−J were derived following enrichment of adducts by butanol extraction. Panels A and B represent two smoker lung DNA with varying degree of adduction. DNA used in panel C was the same as used in panel A, except that it was analyzed after mixing with calf thymus DNA adducted with PAH diolepoxides listed below. Lung DNA in panel G was the same as in panel B, except that the adducts were enriched by butanol extraction. DNA in panel H represents calf thymus DNA adducted with indicated arylamines. Following enzymatic digestion of DNA (10 μg), adducts were enriched by either treatment with nuclease P1 or extraction with butanol. Enriched adducts were labeled with molar excess of [γ-^{32}P]ATP in the presence of T4 polynucleotide kinase. The solvent system I (urea-based solvents both in D3 and D4) and system II (urea-based solvent in D3 and isopropyl alcohol/ammonium hydroxide in D4) are described in detail in text. The figure was prepared by scanning autoradiograms resulting from exposure of chromatograms to Cronex 4 X-ray films at −80 °C for 24 h. Reference adducts used were as follows: spots 1−4 represent adducts resulting from *anti*-diolepoxides of benz(*a*)anthracene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, and dibenz(*a*,*h*)anthracene, respectively; spots 5, 6, and 7 represent the main adducts resulting from *N*-hydroxy derivatives of 4-aminobiphenyl, 2-aminofluorene, and *N*′-acetylbenzidine, respectively.

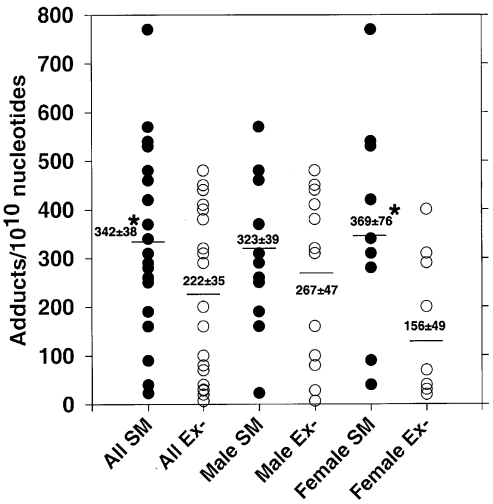

Figure 2. Gender- and smoking status-associated differences in DNA adduct levels in human lung tumors. Data were derived from nuclease P1-mediated ^{32}P-postlabeling assay using high-salt, high urea-based TLC, as described in Figure 1. Data are represented as the mean ± standard error, with each data point denoting adduct levels for individual specimen.

adduct DRZs together with distinct adduct spots in urea-based solvents (Figure 1G), but again, no adducts were detected when D4 development was performed in dilute ammonium hydroxide-based solvent system (Figure 1I).

Measurement of the adduct DRZ radioactivity revealed significantly higher adduct levels in the current smokers (n = 24) compared to ex-smokers (n = 22) (342 ± 38 versus 222 ± 35 adducts/10^{10} nucleotides; P < 0.026) (Figure 2). Adduct levels in the never-smokers (n = 3) (217 adducts/10^{10} nucleotides) were not different than the ex-smokers. Comparison of

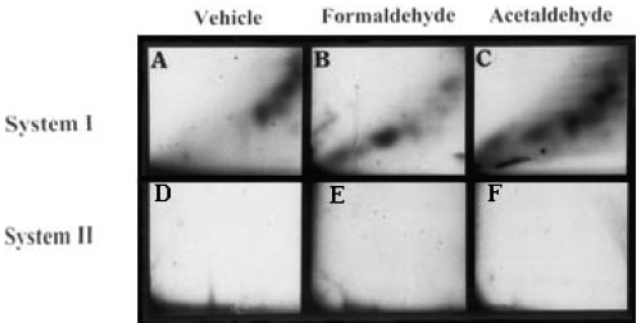

Figure 3. Representative ^{32}P-adduct autoradiograms from calf thymus DNA alone (A and D) and DNA reacted with formaldehyde (B and E) and acetaldehyde (C and F). Adducts were analyzed by nuclease P1-mediated ^{32}P-postlabeling assay and chromatographed using the solvent system I (A−C) and system II (D−F) described in the legend of Figure 1 and the text. Chromatograms were exposed to Cronex 4 X-ray films at −80 °C for 24 h and were reproduced by scanning the autoradiograms.

smokers and ex-smokers in each gender group showed that female current smokers (n = 11) had significantly higher levels of DNA adduction than ex-smokers (n = 9) (369 ± 76 versus 156 ± 49 adducts/10^{10} nucleotides; P = 0.02). However, no significant differences were detected between male current smokers (n = 13) and ex-smokers (n = 13) (323 ± 39 versus 267 ± 47 adducts/10^{10} nucleotides, respectively; P = 0.37). Further statistical analysis revealed that male ex-smokers possessed marginally (P = 0.10) higher adduct levels than the female counterparts (267 ± 47 versus 156 ± 49 adducts/10^{10} nucleotides). One female smoker lung tissue was highly adducted (2930 adducts/10^{10} nucleotides); this value was excluded from the mean value presented in Figure 2. Furthermore, an 8−20-fold interindividual variability was observed in the adduct levels, irrespective of the smoking status.

To identify the chemical nature of the smoke-associated adducts, selected lung DNA samples were analyzed after mixing with calf thymus DNA containing PAH-derived adducts at a level of approximately 1 adduct/10^8 nucleotides, using solvent system I (urea-based solvents in both D3 and D4 directions) and system II (urea-based solvent in D3 and ammonium hydroxide-based in D4). In solvent system I, reference PAH adducts migrated together with lung DNA adducts in the form of DRZ (Figure 1C), but well-resolved, discrete reference adduct spots were observed in the ammonium hydroxide-based solvent, with no additional adducts associated with smoker lung DNA (Figure 1F). DNA adducts derived from various PAHs were characterized and numbered appropriately as BADE (no. 1), BFDE (no. 2), BPDE (no. 3), and DBADE (no. 4) (Figure 1C,F). AA-derived DNA adducts migrated in the chromatographic region where smokers' lung DNA adducts migrated in solvent system I (Figure 1H), but unlike smoker lung DNA adducts which were lost in solvent system II (Figure 1I), the AA-DNA adducts were observed as well-resolved, distinct spots (Figure 1J). Under the experimental conditions used, reference DNA adducts as low as 5 adducts/10^{10} nucleotides were detectable after prolonged autoradiographic exposure. These data suggest an absence of PAH- and AA-derived DNA adducts in the smoker lung DNA samples analyzed.

In attempts to explore the origin of smoke-associated adducts, analysis of formaldehyde- and acetaldehyde-modified DNA by ^{32}P-postlabeling showed typical adduct DRZs in the solvent system I (Figure 3B,C), compared with vehicle treatment (Figure 3A). However, when adducts were resolved in ammonium hydroxide-based solvent in D4 (system II), the DRZs disappeared from the chromatogram (Figure 3D−F). This chroma-

tography behavior of the aldehyde-derived DNA adducts is similar to that of the cigarette smoke-associated DNA adducts.

Discussion

DNA damage as reflected by the formation of adducts is believed to be an essential step in the initiation of the carcinogenic process and has been positively correlated with the mutation frequency and development of cancer (*8*). Several investigators have reported the presence of BP- and 4-ABP-derived DNA adducts sequestered in adduct DRZs in different human tissues (*9*−*12*, *20*). As yet, the complete and true chemical nature of the DRZs is not known, although they are found at higher levels in smokers' tissues and have been implicated in tobacco smoke carcinogenesis (*21*). Results from the present study and from our earlier experimental studies (reviewed in ref *22*) prompted us to suggest that smoking-associated DNA adducts may not be derived from typical PAHs or AAs, normally present in tobacco smoke. These adducts are also unrelated to polar oxidative adducts such as 8-oxo-deoxyguanosine and cyclic DNA adducts resulting from lipid peroxidation products, since such adducts are lost onto paper wick during the chromatographic manipulations. On the basis of the chromatographic resemblance, our data tend to suggest that the smoke-associated DNA adducts may originate, at least in part, from aldehydes such as formaldehyde and acetaldehyde, which are present in significant quantities in cigarette smoke.

One of the limitations of the ^{32}P-postlabeling assay is its inability to identify DNA adducts in the absence of reference adducts of known chemical nature. The appearance of DNA adduct spots on chromatograms depends on their mobilities and can be variable depending on the choice of chromatography solvents and the nature of the chromatographic matrix, for example, cellulose and polyethyleneimine. A complex mixture like tobacco smoke, which contains over 4000 compounds, can potentially generate numerous unidentifiable DNA adducts in the tissues (*7*). Most studies have, however, focused on common environmental carcinogens, like PAHs and AAs, even though aldehydes, catechols, free radicals, and others represent a significant portion of inhaled tobacco smoke constituents (*7*). BP- and 4-ABP-related DNA adducts reported in smokers were identified mostly by ^{32}P-postlabeling in conjunction with high-salt, high-urea TLC. Dilute ammonium hydroxide-based solvents were introduced, which can resolve adduct DRZs into discrete spots (*17*) and allow identification of adducts derived from diverse classes of compounds, including PAHs and AAs.

To obtain a better understanding of the nature of smoking-associated DNA adducts, select lung DNA samples from human smokers were analyzed using ammonium hydroxide- and conventional urea-based solvents, in the presence and absence of PAH- and AA-derived reference adducts. As expected, smoker lung DNA samples, with and without reference adducts, produced prominent adduct DRZs in urea-based solvents. However, when the same samples were analyzed in ammonium hydroxide-based solvent as one of the eluting solvents, they did not yield any adducts, but the same samples mixed with reference adducts produced discrete spots of individual reference adducts. These observations clearly suggested that the DRZs produced by the same smoker lung DNA in urea-based solvents do not contain typical PAH- and/or AA-derived adducts. Our previous finding that BP-derived DNA adducts were undetectable in cigarette smoke-exposed rodent tissues is consistent with the above observations (*13*−*15*).

This conclusion is supported by a recent study in which both vapor phase and whole environmental tobacco smoke were equally effective in producing lung tumors in the A/J mice (*16*). This study also showed that chemopreventive agents that inhibited model carcinogen-induced lung tumors failed to protect against whole smoke-induced lung tumors. These data further support our conclusion that agents other than PAHs may be involved in smoke carcinogenesis.

The discrepancy over the presence of BPDE−DNA adducts in smokers' lung tissues in this report and other studies seems to be method-oriented, particularly the chromatography conditions. van Schooten et al. (*23*) performed co-chromatographic experiments with reference BPDE−DNA adducts in only urea-based solvents as opposed to our present and previous studies (*13*−*15*) where we utilized multiple solvents, including ammonium hydroxide-based solvents in co-chromatography experiments.

An exhaustive analysis of published data on the presence of BP-derived DNA adducts in human tissues by Boysen and Hecht (*24*) demonstrates that 39% of 705 human tissues showed detectable levels of BPDE−DNA adducts. These measurements were based on tetrols from BPDE. The BPDE−DNA adducts were detected in 45% smokers, 33% ex-smokers, 52% non-smokers, 39% of occupationally exposed individuals, and 34% of environmentally exposed people. The data further indicated that BPDE−DNA adducts in smokers' tissues are not consistently different that in nonsmokers' tissues and that due to the environmental ubiquity of BP, it cannot be assumed that BPDE−DNA adducts are present in human smokers' tissues (*24*).

Several studies have identified PAH- and AA-derived protein adducts in human smokers as well as smoke-exposed rat tissues (*25*, *26*). The undetectability of DNA adducts from these carcinogens in human smokers in the present study and cigarette smoke-exposed rat tissues in our previous studies (reviewed in ref *22*) suggests that electrophilic metabolites of PAHs and AAs present in cigarette smoke may be consumed by the enormous amounts of cellular proteins before they have a chance to cross the nuclear membrane to react with the DNA.

A potential role of various short- and longer-lived free radicals of cigarette smoke in forming adduct DRZs cannot be ruled out, since cigarette smoke contains high levels of free radicals (10^{14}−10^{16} radicals/puff) in both its gaseous and tar phases (*27*). Findings from the laboratories of Randerath and Phillips reported the detection of intrastrand DNA−DNA cross-links formed by treatment of DNA with hydroxyl radicals (*28*, *29*). Using urea-based solvents, as employed in our studies, these investigators were able to separate intrastrand cross-links on the chromatograms, suggesting that the smoke-related DNA adducts present as adduct DRZs may chromatographically behave as certain DNA−DNA cross-link products. DNA adducts produced by reaction with formaldehyde and acetaldehyde in vitro showed adduct DRZs as found in smokers' lung DNA when chromatographed in urea-based solvents but, when chromatographed in ammonium hydroxide-based solvent did not yield any detectable adducts. Additionally, cross-link adducts prepared by reaction of rat lung nuclei with a known oxidant, potassium dichromate, also behaved chromatographically similarly by producing adduct DRZs in urea-based solvents and were abolished in ammonium hydroxide-based solvents (Arif, J. M. and Gupta, R. C., unpublished data). Taken together, our data strongly suggest that cigarette smoke-related adduct DRZs may represent DNA−DNA and/or DNA−protein cross-links which can result from either free radicals generated at the target site from catechols and/or from smoke carcinogens such as aldehydes and butadiene, all of which are present in levels that are orders of magnitude

higher than the levels of lipophilic polyaromatic carcinogens (*7*).

In conclusion, our study clearly demonstrates higher levels of adduct DRZs in lung tissues of current smokers than ex- or never-smokers, consistent with the literature, but suggests that the smoke-associated adducts are unrelated to typical polycyclic aromatic hydrocarbons and aromatic amines. These DNA adducts are implicated as products derived by cross-linking agents such as aldehydes, butadiene, and catechols, which are present in cigarette smoke in levels that are orders of magnitude higher than the typical polyaromatic carcinogens. Our findings clearly warrant a systematic study to determine the role of the constituents in tobacco smoke other than typical PAHs, since it would not only establish an association with tobacco smoke lung carcinogenesis but also turn the focus of preclinical and clinical intervention studies with appropriate chemopreventive agents.

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