An Improved Fluorometric Assay for Dosimetry of Benzo(a)Pyrene Diol-Epoxide-DNA Adducts in Smokers' Lung: Comparisons with Total Bulky Adducts and Aryl Hydrocarbon Hydroxylase Activity

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ABSTRACT

An improved high-performance liquid chromatography/fluorometric assay has been established to quantitate the benzo(a)pyrene (BP) tetrots released after acid hydrolysis of lung DNA from lung cancer patients, so that the formation of benzo(a)pyrene diol-epoxide-DNA adducts can be measured. The r-7,c-10,t-8,f-9-tetrahydroxy-7,8,9,10-tetrahydro-BP isolated by high-performance liquid chromatography was determined by fluoromicroscopy in two different solvent systems and fluorescence spectroscopy. This assay has a detection limit of 2 pg of r-7,c-10,t-8,f-9-tetrahydroxy-7,8,9,10-tetrahydro-BP, requires 100–500 μg of DNA, and can measure 1 adduct/108 unmodified nucleotides. As this assay does not use immunoaffinity chromatography or solvent extraction, it allows a >90% recovery of benzo(a)pyrene diol-epoxide-DNA adducts. This procedure has been tested on 13 DNA samples prepared from nontumorous lung parenchyma taken from lung cancer patients at surgery and revealed the presence of DNA adducts of the anti-benzo(a)-pyrene diol-epoxide in 9 of 11 samples from smokers and in 2 of 2 exsmokers. In only two samples from smokers the formation of adducts derived from syn-benzo(a)pyrene diol-epoxide was detected. A 15-fold variation in DNA adduct level was found in 11 of 13 DNA samples, with a range of 0.6–9.9 adducts of benzo(a)pyrene diol-epoxide/108 nucleotides. In samples containing both anti- and syn-benzo(a)pyrene diol-epoxide-DNA adducts, the anti/syn adduct ratio is 2:1. A highly significant correlation was found between pulmonary microsomal aryl hydrocarbon hydroxylase activity and the level of benzo(a)pyrene diol-epoxide-DNA adduct (r = 0.91; P < 0.001; n = 13). A crude linear correlation between the amounts of these adducts and those of bulky DNA adducts determined by 32P-postlabeling assay was observed in the same samples (r = 0.78; P < 0.02; n = 13). Thus this highly sensitive and specific procedure is suitable for measuring benzo(a)pyrene diol-epoxide-DNA adducts in human tissues from environmentally exposed subjects and could be adapted to measure polycyclic aromatic hydrocarbons other than BP.

INTRODUCTION

Tobacco smoke contains the carcinogen benzo(a)pyrene that is considered, together with other carcinogens, to contribute to human lung cancer risk in smokers (1, 2). Although the presence of BP in human lung was observed as early as 1959 (3), the detection of BP-DNA adducts in human lung has only been more recently reported (see below). The formation of BP diol-epoxide-DNA adducts (Fig. 1a) is now considered to be the critical event in tumorigenesis by BP, being responsible for the mutational activation of ras protooncogenes in mouse lung tumors (4). As this adduct may also cause activation of ras protooncogenes in human large cell carcinomas and adenocarcinomas of the lung, sensitive and specific methods to measure the levels of BPDE-DNA adducts in human lung are needed.

So far three analytical methods have been used to quantify carcinogen-DNA adducts in human lung; however, each has some limitations, (a) immunological assays (5–7); (b) 32P-postlabeling assays (6, 8–14); and (c) HPLC-linked SFS (7). Immunological and 32P-postlabeling assays detect broad spectra of PAH-DNA adducts; the antibody designed to recognize anti-BPDE-DNA adducts shows various levels of cross-reactivity with structurally related congeners, while 32P-postlabeling of the adducts gives a number of spots of unidentified nature, and overlap between such spots is liable to interfere with adduct identification especially with quantitation (5–14). SFS has been used to examine BPDE-DNA adducts in human lung (7) but only allows the presence of BPDE-DNA to be detected. Thus, these analytical methods cannot unequivocally identify and quantify BPDE-DNA adducts in human lung, mainly because the presence in human samples of multiple DNA adducts arising from complex exposures that confound simple assay systems, the low levels of adducts, and the multiplicity of adducts formed from a given carcinogen challenge the detection limits of these techniques. Recently, the presence of BPDE-DNA adducts in human lung was detected by using immunoaffinity chromatography followed by HPLC/SFS (15). The recovery of the adducts, however, ranged from 26 to 66% for the overall procedure, including immunochromatography, enzyme and acid hydrolysis, alkalai and solvent extraction, HPLC, and SFS. Another recent study used rabbit polyclonal antibodies for the isolation of BPDE- and BPDE-DNA adducts on the basis of adducts formed from a given carcinogen challenge the detection limits of these techniques. Recently, the presence of BPDE-DNA adducts in human lung was detected by using immunoaffinity chromatography followed by HPLC/SFS (15). The recovery of the adducts, however, ranged from 26 to 66% for the overall procedure, including immunochromatography, enzyme and acid hydrolysis, alkalai and solvent extraction, HPLC, and SFS. Another recent study used rabbit polyclonal antibodies for the isolation of BPDE- and BPDE-DNA adducts on the basis of adducts formed from a given carcinogen challenge the detection limits of these techniques. Recently, the presence of BPDE-DNA adducts in human lung was detected by using immunoaffinity chromatography followed by HPLC/SFS (15). The recovery of the adducts, however, ranged from 26 to 66% for the overall procedure, including immunochromatography, enzyme and acid hydrolysis, alkalai and solvent extraction, HPLC, and SFS. Another recent study used rabbit polyclonal antibodies for the isolation of BPDE- and BPDE-DNA adducts on the basis of adducts formed from a given carcinogen challenge the detection limits of these techniques.
base of HPLC/fluorescence detection of BP-tetrols released after acid hydrolysis of DNA-adducts (Fig. 1b); (b) to increase the recovery of the BPDE-DNA adducts during their isolation; and (c) to apply the resulting methods to a series of human lung samples to answer the following questions. (d) Does formation of BPDE-DNA adducts correlate with the levels of aromatic/bulky DNA adducts quantified by $^{32}$P-postlabeling assay? (e) Does the formation of BPDE-DNA adducts correlate with microsomal AHH activity in the same human lung sample?

**MATERIALS AND METHODS**

Chemicals. The enzymes for DNA purification and hydrolysis, proteinase K, RNase A, DNase I, alkaline phosphatase, and nuclease P1 were obtained from Boehringer Mannheim Biochemicals (Germany). HPLC-grade water (Rathburn Chemicals Ltd., Walberburn, United Kingdom), ethanol, acetonitrile, and methanol (Lichrosolv and Prep- solv), hydrochloric acid (0.5 n Tritisol), and NaOH (1 n Tritisol) were purchased from E. Merck, Darmstadt, Germany.

Human Lung Specimens. Human lung DNA samples analyzed in this study (except for 2 exsmokers; Table 1) were chosen from the surgical lung parenchyma samples previously analyzed in a comparative study on pulmonary DNA adduct levels and AHH activity (13). Detailed information on the study subjects, smoking habits, measurement of AHH activity, DNA extraction, and quantitation of DNA adducts by $^{32}$P-postlabeling assay is given in Ref. 13. In brief, microsomal AHH activity was determined by a fluorimetric assay with a detection limit of 0.01 pmol 3-hydroxy-BP/min/mg protein. Quantitation of DNA adducts was carried out by $^{32}$P-postlabeling assay after enrichment of adducted nucleotides, using treatment with nuclease P1. After purification overnight onto a column with the use of a polyethyleneimine cellulose thin layer chromatography plate, adducts were chromatographed on a two-dimensional polyethyleneimine cellulose thin layer chromatography plate, using the transfer chromatography technique of Luet et al. described in Geneste et al. (13). The quantitation of normal nucleotides and calculation of the adduct level were based on the assumption that the adducts were completely resistant to 3'-dephosphorylation by nuclease P1. The DNA samples used in this study were reprecipitated at least three times with ethanol, until no fluorescence was present in HPLC chromatograms of ethanol washings at the retention times of BP-tetrols.

Purification of Hydrochloric Acid Used for Hydrolysis of Human Lung DNA Samples. This procedure is very important for a correct HPLC run. Commercial hydrochloric acid, even of a high degree of purity, contains some fluorescent impurities, particularly one which has the same retention time on HPLC as that of BP-tetrol 1-1. To eliminate all fluorescent products from HCl (with the possible exception of those eluting during the first 5 min of a HPLC run), we proceeded as follows: an aliquot of 0.1 n HCl was heated for 4 h at 90°C and extracted with ethyl acetate until no fluorescent peaks appeared in the HPLC run after neutralization with 1 n NaOH to pH 7-7.5.

Analytical Procedures. The method used in this study is a modification of that described previously for analysis of BP-tetrols released from DNA adducted with BPDE (17, 18). Because of the low levels of BP-tetrols being measured, a number of rigorous precautions were taken to minimize fluorescent contamination that could interfere with the analysis of BP-tetrols; these include the use of a dedicated laboratory location, silanized vials, and clean tubing, HPLC syringes, and other equipment (all washed with methanol). Three quantities of each lung DNA sample (125, 250, and 500 n g DNA) were dissolved in 1 ml of water and analyzed according to the analytical scheme outlined in Fig. 2. The first step, carried out in the same flask, consisted of the following four procedures: (a) addition of 0.4 n HCl to bring the final concentration of acid to 0.1 n; (b) hydrolysis of DNA for 4 h at 90°C; (c) neutralization of the solution with 1 n NaOH to pH 7-7.5; and (d) addition of methanol to give a solution of 20% methanol. The second step consisted of concentration of BP-tetrols and their separation on HPLC, as follows. The solution in the first step (~1.2 ml) was loaded onto a precolumn module (Herbert Knauer GmbH, Berlin, Germany) containing 10-µm C18 reverse-phase material equilibrated with 20% methanol for 10 min at a flow rate of 2.0 ml/min. The precolumn was washed with 20% methanol for 10 min at a flow rate of 0.5 ml/min. The concentrated material on the precolumn was then switched with the help of a Valco valve C10V (Vici, Valco Instruments Co., Inc., Switzerland) to a Lichrosorb RP prepacked 5-µm C18 reverse-phase analytical column (250 x 4.0 mm, Herbert Knauer GmbH). The column was eluted isocratically with 55% methanol in water at a flow rate of 1.0 ml/min by using a Beckman Model 114 delivery system. A Perkin-Elmer S40 fluorescence detector (Model LS 40, Perkin-Elmer, Ltd., Beaconsfield, Buckinghamshire, United Kingdom) linked to a Hewlett-Packard HP 3394A integrator was used for peak detection. The excitation wavelength was set at 344 nm and the fluorescence emission wavelength at 398 nm. The sensitivity of the detector was fixed at factor 70. In some samples the fractions containing the BP-tetrol 1-1 were collected, evaporated to dryness under nitrogen, redissolved in a minimal amount of methanol (50 µl) and further analyzed by HPLC, using an acetonitrile/water gradient (5 min with 20% acetonitrile/water, followed by a linear gradient to 100% acetonitrile in 60 min at a flow rate of 0.8 ml/min). The eluate was monitored for fluorescence as described above. The fluorescence corresponding to the eluted peaks of BP-tetrol 1-1 and BP-tetrol II-2 was integrated and the levels of BP-tetrols in the samples were determined by comparison with a standard curve generated from fluorescence heights of an authentic tetrol standard at the

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* NLC, nonmalignant lung disease such as emphysema; LC, lung cancer that includes undifferentiated large cell carcinoma (ULCC); adenocarcinoma (AC); squamous cell carcinoma (SCC); undifferentiated small cell carcinoma (USCC); and bronchial adenosquamous carcinoma (BAC).

(2) Number of cigarettes smoked per day x years of smoking/20.

(3) pmol 3-hydroxybenzo[a]pyrene/min/mg protein.

* Measured by fluorescence, this study; the amounts of samples 1 and 4 represent the sum of anti- and syn-BPDE-DNA adducts (2:1 ratio), while the adducts of all other samples are derived from anti-BPDE.

* ND, not detected.

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The difference in the retention times between BP-tetrol I-1 eluting first, and the other three BP-tetrols (II-1, I-2, and II-2; see Fig. 1) was as follows: 1.94 min (for I-1), 3.70 min (for I-2), and 11.10 min (for II-2), respectively. The variations in the calibration curve did not exceed 5% during an experiment. The values thus obtained for BP-tetrols correspond to the quantities of BPDE-DNA adducts detected in human lung parenchymal samples (Fig. 1).

RESULTS

Testing the Assay with BP-Tetrol I-1, BP-Tetrol II-2, and Anti-BPDE-modified Calf Thymus DNA. Before applying the assay to human lung DNA samples, we tested it by using known quantities of BP-tetrol I-1, BP-tetrol II-2, and BPDE-modified calf thymus DNA. In the first test, samples of the two BP-tetrols were taken through the procedure outlined in Fig. 2. The fluorescence response was linear with the amount of BP-tetrol and the variability of triplicate tests did not exceed 5% (data not shown). The lower detection limit with the instrumentation used was 2.0 pg of BP-tetrol I-1. In a second test, we spiked 300-μg aliquots of acid-hydrolyzed calf thymus DNA with 2-34 pg of BP-tetrol I-1. Analysis of these DNA samples revealed a linear relationship (Fig. 3) between the amount of BP-tetrol I-1 spiked and the amount measured (r = 0.97). In a third test, the variability of the method for a given sample was tested by spiking 1.5 mg of hydrolyzed calf thymus DNA with 50 pg of BP-tetrol I-1. This sample was split into five equal portions (each containing 10 pg tetrol), and analysis of each aliquot for BP-tetrol I-1 indicated the presence of 9.6 ± 0.2 pg (SD). These three tests were also performed with BP-tetrol II-2 and results were similar to those obtained with BP-tetrol I-1. These results show the high precision of the method. In a fourth test, we used calf thymus DNA adducted with anti-[3H]BPDE. This assay shows good agreement between the fluorescence and the radioactivity associated with BP-tetrol I-1 with >90% recovery (Fig. 3). These results demonstrate a distinct improvement when compared with previous methods, where 26-66% recovery of BP-tetrol I-1 were obtained when a low level of anti-[3H]-

BPDE-modified DNA was analyzed by a method including immunofinity chromatography enzyme and acid hydrolysis and solvent extraction steps (15).

Analysis of BP-Tetrols Released from Human Lung DNA. BP-tetrols released after hydrolysis of human lung DNA and corresponding to BPDE-DNA adducts formed in human lung were analyzed by using reverse-phase HPLC to characterize the diastereoisomeric form of BPDE formed. Representative profiles of the hydrolys products from two different samples (patients 2 and 12) are shown in Fig. 4. The fluorescent product found on HPLC run (I-1) corresponds in its retention time to the standard BP-tetrol I-1. Two chromatograms of each sample obtained with 2-fold different DNA concentrations [125 (b,b') and 250 (c,c') μg DNA] showed corresponding 2-fold differences in the fluorescence response and the amounts of BP-tetrol I-1 (1.6 and 3.3 pg for patient 12 and 3.5 and 7 pg for patient 2) (Fig. 4, b and c, b' and c', respectively). The background fluorescence of the hydrolysate of calf thymus DNA was presented on Fig. 4, a and a'.
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The presence of two other products [unknown (X') and II-2, Fig. 5 C] was observed in two samples (samples 1 and 4) in contrast to all other samples (Fig. 5B). The product II-2 had the same retention time on HPLC as the BP-tetrol-II-2 derived from hydrolysis of syn-BPDE-DNA adducts (Fig. 1b). The products I-1 and II-2 isolated isocratically with 55% methanol in water (Figs. 4 and 5) were rerun on HPLC by using an acetonitrile:water gradient. Again, the retention times of the two products (I-1 and II-2) and BP-tetrol standards were similar (data not shown). Furthermore, the product I-1 (Figs. 4 and 5) was collected from several HPLC runs, thus obtaining a quantity of material that by its fluorescence intensity was equivalent to 132 pg of BP-tetrol I-1. Using a Perkin-Elmer S40 fluorescence detector (see legend to Fig. 6) the fluorescence excitation spectrum (emission at 400 nm) (Fig. 6A) and synchronous fluorescence spectra (Fig. 6B) of this product (I-1) were performed as indicated in legend to Fig. 6; they were identical to those of authentic BP-tetrol I-1. The specific synchronous fluorescence signal occurs at 344 nm, identical to that of authentic standard BP-tetrol I-1. Due to the small quantity of BP-tetrol II-2, its fluorescence excitation spectra could not be recorded. These results tentatively indicated the formation of anti- and syn-BPDE-DNA adducts in human lung. While in all positive cases the formation of anti-BPDE adducts was observed, the occurrence in 2 of 13 cases of BP-tetrol II-2 indicates the involvement of syn-BPDE in DNA adduct formation. In these two cases, the anti/syn-adduct ratio was 2:1. The use of gas chromatography-mass spectrometry would be of great help to confirm the identity of the BP-tetrols derived from human lung DNA, but we did not obtain enough material to perform this

Fig. 4. Reverse-phase HPLC profiles of the BP-tetrols formed after acid hydrolysis of DNA (2-fold different concentrations, 125 and 250 µg) of two human lung samples (samples 2 and 12). a,d, calf thymus DNA (250 µg); b,c, human lung DNA patient 12, 125 µg DNA (b) and 250 µg DNA (c); f', human lung DNA, patient 2, 125 µg DNA (f') and 250 µg DNA (c'). The procedures for the isolation and separation of tetrol 1-1 are given in "Materials and Methods." The symbols assigned to each peak correspond to compounds as described in the footnote 4. BP tetrol I-1, tetrol derived from anti-BPDE.

Fig. 5. Reverse-phase HPLC profiles of the BP-tetrols formed after acid hydrolysis of DNA samples. The procedure is outlined in Fig. 2. (A) 250 µg calf thymus DNA; (B) 250 µg human lung DNA (patient 2); and (C) 250 µg human lung DNA (patient 1). The procedures for the isolation and separation of BP-tetrols are given in "Materials and Methods." The symbols assigned to each peak correspond to compounds as described in footnote 4. BP tetrol I-1, tetrol derived from anti-BPDE; BP tetrol II-2, tetrol derived from syn-BPDE; X, unknown.

Fig. 6. Fluorescence excitation spectra (A) and synchronous fluorescence spectra (B) of BP-tetrol-II-2 isolated from human lung DNA samples. The excitation spectra were recorded from 230 nm to 360 nm and the synchronous fluorescence spectra were recorded at a wavelength difference of 34 nm between the excitation and the emission monochromators (SFS).

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analysis, as was done earlier for BP-diol epoxide DNA adducts in human placenta and peripheral blood lymphocytes (19, 20).

Intersubject Variations in BPDE-DNA Adducts; Relationship with AHH and Bulky Adducts by 32P-Postlabeling Procedure. Our assay was applied to all 13 lung DNA samples from subjects with lung cancer undergoing surgical intervention. The variation between triplicate or quadruplicate tests with each sample did not exceed 6%. The amounts of BPDE-DNA adducts varied by a factor of 15 (0.6-9.9 adducts/10⁸ nucleotides) among the individual samples (Table 1). The amounts of samples 1 and 4 represent the sum of anti- and syn-BPDE-DNA adducts (2:1 ratio), while the adducts of all other samples are derived from anti-BPDE. BPDE-DNA adducts were not detected in two individuals. Interestingly, the highest amounts of BPDE-DNA adducts and the presence of syn-BPDE-DNA adducts were observed in the two patients with the highest AHH activity and highest formation of DNA adducts as detected by 32P-postlabeling assay (patients 1 and 4; Table 1). In our group of subjects there was crude linear correlation (r = 0.78; P < 0.02; n = 13) between the level of BPDE-DNA adducts and those found by the 32P-postlabeling procedure, which is not surprising in view of the complexity of agents in tobacco smoke that give rise to bulky/aromatic DNA adducts. In two smokers no BPDE-DNA adducts were detected, although 32P-postlabeling assay revealed the presence of 3 and 6 adducts/10⁸ nucleotides, respectively. A positive linear correlation was observed in this study between BPDE-DNA adduct levels in smokers' lung and microsomal AHH activity in the same sample (r = 0.91; P < 0.001; n = 13), but the limited number of subjects in our study is emphasized (Fig. 7). We did not find a correlation between BPDE-DNA adduct levels and tobacco consumption measured either as pack-years (r = −0.12) or as cigarettes per day (r = −0.06) as listed in Table 1.

DISCUSSION

A few measurements of DNA adducts in human lung have been reported (5–15). Measurement of adducts in lung cancer patients showed levels of polycyclic aromatic hydrocarbon adducts of 2–134 adducts/10⁸ nucleotides by enzyme-linked immunoassorbent assay. 32P-Postlabeling analysis of unidentified adducts in human lung DNA gave levels of between 2 and 35 adducts/10⁸ nucleotides (6, 8–11, 13) and human bronchial epithelium gave levels of 1–10 adducts/10⁸ nucleotides (12). Studies using less chemical-specific and sensitive approaches have suggested the presence of BPDE-DNA adducts in human lung (5–7). Using immunoassays that used polyclonal and monoclonal anti-BPDE-DNA antibodies, ~40% of the samples were found to be positive. However, quantitation of BPDE-DNA adducts by immunoassay is complicated by the fact that the antibodies have been found to react with a relatively broad range of PAH-DNA adducts and do not allow the measurement of low levels of biologically formed BPDE-DNA adducts. Using 32P-postlabeling of DNA adducts, Van Schooten et al. (6) detected BPDE-DNA adduct spots in human lung DNA (0.2–4 adducts/10⁸ nucleotides), representing approximately 1 in 10 of all detected adducts. A subsequent report has described the use of eight analytical steps, including immunoaffinity chromatography, enzyme and acid hydrolysis, solvent extraction steps, and HPLC/SFS for dosimetry of BP-DNA adduct levels in lungs of autopsy subjects (15). The data show that BPDE-DNA adducts were present in 6 of 25 human lung samples analyzed, at levels between 1 and 40 adducts in 10⁸ unmodified nucleotides, and 19 samples were below the limit of detection. In 6 of 11 determinations using the 6 positive samples, the levels of BPDE-DNA adducts were in the range found for the total DNA adducts measured by 32P-postlabeling assay in other studies (6, 8–13); the use of immunoaffinity chromatography led to >50% loss of DNA adducts as shown by others for low levels of modification of DNA (16). The levels of BP-tetrols measured by SFS reported in the literature cannot be interpreted as actual levels of BP-tetrols, because other substances contribute to the fluorescence. In fact, this is a serious shortcoming of SFS; it is not an analytical technique with sufficiently high resolving power to determine a single compound in a complex mixture. The application of HPLC before SFS or fluorescence detection of BP-tetrols greatly improves the assay but does not eliminate all the hydrochloric acid-derived fluorescent impurities having the same retention time as BP-tetrol 1-1. The previously used HPLC/fluorescence assay (17, 18) is not applicable to human lung samples for the following reasons: (a) the fluorescent impurities from HCl and other sources were not eliminated, leading to a high background fluorescence with many other peaks; (b) the presence on HPLC chromatograms of fluorescent products unrelated to BP-tetrols but having the same retention time as BP-tetrol 1-1. It is only appropriate for highly modified BPDE-DNA which releases ~100 times more BP-tetrols than human lung DNA.

Our assay eliminates these limitations and allows the measurement of the low levels of BPDE-DNA adducts in human lung. The improved HPLC/fluorescent assay comprises two analytical steps and permits the quantitation of BPDE-DNA adducts formed in vivo in human lung with a recovery >90% of the total adducts. We have thus been able to show unequivocally (a) the formation of anti- and syn-BPDE-DNA adducts in lung samples from smokers, and (b) a highly significant positive correlation between the lung microsomal AHH activity and the formation of BPDE-DNA adducts. This suggests that in human lung BPDE-DNA adducts are mediated predominantly by cytochrome P-450 1A1 as a rate-limiting step, while in human liver other cytochrome species are involved in oxidative BP metabolism (21). The correlation that we have seen is surprising in view of the multiplicity of cytochrome P-450 450 species that are possibly involved in BP metabolism in the lung. Further
investigations with the use of a large number of subjects are necessary before firm conclusions can be drawn, but our results suggest that BPDE-DNA adducts in smokers' lung are predominantly formed, as a rate-limiting step, via a cytochrome P-450 1A1-mediated reaction. A previous study in this laboratory has also shown a crude linear correlation \((r = 0.69; P < 0.01; n = 19)\) between lung DNA adduct levels measured by \(^{32}\)P-postlabeling assay and lung microsomal AHH activity in smokers (13).

The \(^{32}\)P-postlabeling assay revealed that, in addition to BP-adducts in human lungs of smokers, there is a large number of unidentified adducts, presumably arising from PAH. The tetrols released from these adducts were not detected by our method, demonstrating its selectivity for BP-tetrols. Some possible reasons for this selectivity are: (a) the amounts of the released hydrolysis products, e.g., other PAH-tetrols, are not detectable by this assay; (b) there is a difference in the fluorescence response at a given excitation and emission wavelength; and (c) BP-tetrols are known to exert stronger fluorescence than other PAH-tetrols; (d) it is conceivable that some PAH adducts in human lung DNA are rapidly lost during analysis due to their instability, while BPDE-DNA adducts are known to be relatively stable. This could explain, in part, the discrepancy in the relationship between adduct levels measured by \(^{32}\)P-postlabeling and those measured by the fluorescence method. The latter gives values which are frequently 50% or more of the total adducts measured by the much less selective \(^{32}\)P-postlabeling procedure. It is also known that different carcinogen adducts have different \(^{32}\)P-labeling efficiencies; therefore, we believe that results from our fluorescence assay reflect more true values of BP-DNA adducts in keeping with a recent paper (6): BP-diol-epoxide DNA adducts when quantified by \(^{32}\)P-postlabeling as individual spots represented 10–70% of the total bulky aromatic DNA adducts in smokers' lung.

In conclusion, our new assay is simple and sensitive, and does not require immunochromatography and solvent extraction (14). However, rigorous precautions must be taken to eliminate any foreign fluorescence that might interfere with that of BP-tetrols. This methodology could also be adapted to measure other PAH-DNA adducts.

With our improved method to quantify very low levels of BP-DNA adducts it will be possible to examine their dependence on a metabolic phenotype related to cytochrome P-450 and glutathione-S-transferase polymorphism; moreover, the quantitative relationship between BP-DNA adducts in human lung and lung cancer risk in smoking individuals can now be investigated.

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An Improved Fluorometric Assay for Dosimetry of Benzo(a)pyrene Diol-Epoxide-DNA Adducts in Smokers' Lung: Comparisons with Total Bulky Adducts and Aryl Hydrocarbon Hydroxylase Activity

Kroum Alexandrov, Margarita Rojas, Olivier Geneste, et al.


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