Detection of Benzo(a)pyrene:DNA Adducts in Human White Blood Cells


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ABSTRACT

Metabolic activation of benzo(a)pyrene (BP) to its ultimate carcinogenic form, 7,8,9,10-tetrahydroxy-1,2-benzanthracene (BPDE), and the binding of BPDE to DNA are important steps in BP carcinogenicity in experimental animals. Since people of certain occupations are exposed to high concentrations of BP, we have used enzyme-linked immunossay and ultra-sensitive enzymatic radioimmunoassay to measure BPDE:DNA adducts in white blood cells from 2 of these occupational groups. Seven of 28 samples from roofers and 7 of 20 samples from foundry workers were positive for BPDE:DNA adducts (range, 2 to 120 fmol BPDE/50 μg DNA). In a group of nine volunteers without these industrial exposures to BP, the two positive DNA samples were from cigarette smokers. Control DNA obtained from human lymphocyte cell line RPMI 1555 was negative. These results indicate that the metabolic activation of BP and formation of BPDE:DNA adducts occurs in humans.

INTRODUCTION

Chemical carcinogens and cocarcinogens are considered to be responsible for many of the cancers of humans (16). Because of the nature of their occupation, certain people more than others are exposed to higher concentrations of carcinogens prevailing in the work environment (8). It is widely accepted that the reaction of carcinogens with cellular macromolecules (especially DNA) is an important event in carcinogenesis (21). Metabolic pathways of carcinogen activation and carcinogenesis:DNA adducts have been identified and quantitated in cultured human tissues and cells experimentally exposed to radiolabeled carcinogens (5, 9, 13, 19, 22). The carcinogen potency of various polycyclic aromatic hydrocarbons shows a fairly good correlation with their ability to form adducts with DNA (4, 17, 21, 23).

Detection of low levels of carcinogen:DNA adducts in exposed individuals is not feasible with conventional scintillation counting, radioimmunoassays, or chromatographic techniques, because the carcinogens in our environment are not generally radiolabeled, and the amount of carcinogen:DNA adducts in exposed individuals are expected to occur in minute amounts (i.e., 1 adduct in 10^7-8 nucleotides). Using rabbit anti-BPDE-DNA antibody (25) and ELISA (7), Perera et al. (24) have recently reported presence of BP:DNA adducts in human lung tissue of patients with cancer of the lung. In order to establish the relationship between exposure and adduct formation in humans in vivo and to monitor such mechanisms in ready available biological materials, we investigated the presence of BP:DNA adducts in the peripheral blood WBC of 2 occupational groups of roofers and foundry workers who are known to be heavily exposed to BP (10, 11). Using USERIA (14, 18), a more sensitive assay than the ELISA system, we now report that BP:DNA adducts can be quantitatively detected in the peripheral WBC of exposed humans.

MATERIALS AND METHODS

From 25 to 40 ml of peripheral blood were obtained from 48 male volunteers who were active in their occupations as roofers or foundry workers. The blood samples were centrifuged at 800 x g for 15 min, and the "buffy coat," containing WBC, were separated. The isolated "buffy coat" was homogenized in 5 volumes of HKM:0.25 M sucrose buffer containing 0.5% Triton X-100 and centrifuged for 10 min at 800 x g at 4°. The pellet was resuspended in 5 ml HKM:sucrose buffer containing 1% sodium dodecyl sulfate and 1 M NaCl. An equal volume of chloroform:isoamyl alcohol (24:1, v/v) was added, and the mixture was vigorously agitated for at least 20 min, followed by centrifugation at 10,000 x g for 10 min. The aqueous epiphase was removed and treated with RNase (100 μg/ml) at 37° for 20 min. Following RNase digestion, 3 volumes of cold ethanol were added to the solution. DNA was removed by winding onto a glass rod. Residual ethanol was evaporated under nitrogen, and DNA was dissolved in water. Purity and quantitation of DNA were determined by absorbance at 260 and 280 nm using a Beckman DU8 spectrophotometer. The final volume of DNA solution was adjusted to 1 mg DNA/ml water and rendered single-stranded by boiling. Single-stranded DNA was then stored at 4° until tested.

Competitive enzyme immunoassays, USERIA and ELISA, were performed on the test DNA samples by using rabbit anti-BPDE-DNA antibody. Polyvinyl U-bottomed 96-well microtiter plates (Dynatech Laboratories, Inc., Alexandria, VA) were coated with unmodified DNA (control) and BPDE-modified DNA (0.114% modification, 1 μg/well for USERIA and 5 μg/well for ELISA). Standard competitive inhibition curves were obtained by mixing serial dilutions of known standard BPDE-DNA with rabbit antisera (Chart 1). Reproducibility of the USERIA was determined by repeatedly performing the assay on different days (Chart 2).

Prior to the assays, the microtiter wells were treated with 200 μl each of 1% horse serum. Rabbit anti-BPDE-DNA antibodies (2.5 x 10^-4 dilution for USERIA and 1 x 10^-4 dilution for ELISA) were diluted in Dulbecco's PBS-Tween (0.05%) containing 1% horse serum. For standard curves, serial dilutions of known standard BPDE-DNA mixed with antisera were added to wells containing BPDE-DNA or unmodified calf thymus DNA. Test DNA (50 μg/ml) was mixed with similar dilutions of antisera and added to wells in the same plate in which standard inhibition was performed. The antigen-antibody mixture was incubated at 37° for 1 hr and washed 5 times with PBS-Tween. Goat anti-rabbit IgG conjugated with alkaline phosphatase was then added (Miles Laboratories Inc.,

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RESULTS

Using USERIA, 14 of 48 tested DNA samples from roofers and foundry workers were found to be positive for BPDE:DNA adducts (Table 1). The commercial calf thymus DNA and DNA extracted from the lymphocyte cell line were negative. Of the 9 volunteer laboratory personnel, 2 were positive.

The smallest amount of adduct detected is 2 femtomoles (fmol)/50 μg, which is equivalent to 1 BPDE:DNA adduct in 7.5 × 10^7 bases. Because of the shape of the standard curve, values less than 2 fmol were considered negative. There was a marked interindividual variation in the levels of adduct (2 to >100 fmol/50 μg). In general, the level of adducts was higher for the roofers than the foundry workers (Table 1). Interestingly, the 2 volunteers who were positive (37 fmol and 47 fmol/50 μg) were also the only known cigarette smokers among the 9 volunteers.

In order to see a dose-response effect and reproducibility of the assay, serial dilutions of DNA (R0106) were tested, and standard inhibition curves were similarly obtained. With increasing dilutions of the test DNA, there was decreasing inhibition, the estimated amount of BPDE being similar (see Table 1). Samples were repeatedly tested by assays performed on different days, and the values were within 20% of one another. Sample R0108 was retested by extracting DNA from a second aliquot of WBC, and the amounts of BPDE:DNA adducts were similar on both occasions.

DISCUSSION

We have used a highly sensitive immunoassay system and a highly specific antiserum to detect BPDE:DNA in human WBC DNA. The sensitivity and reproducibility of USERIA has been well documented, both in previous publications (13, 18) and in this study (Chart 2). Likewise, the specificity of the antiserum has been well tested (26). The antiserum is specific for BPDE:deoxyguanosine and BPDE:DNA and does not recognize free BP, free BPDE tetrol, unmodified deoxyguanosine, unmodified DNA, or 2-acetylaminofluorene-modified DNA.

Since the peripheral blood cells can metabolize BP (1, 3), it is

**Table 1**

<table>
<thead>
<tr>
<th>Sample</th>
<th>% inhibition</th>
<th>fmol/50 μg DNA</th>
<th>Smoking habits (cigarettes/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R0102</td>
<td>43</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>R0105</td>
<td>98</td>
<td>&gt;100</td>
<td>70</td>
</tr>
<tr>
<td>R0106</td>
<td>96</td>
<td>&gt;100</td>
<td>Never</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>R0107</td>
<td>31</td>
<td>2</td>
<td>55</td>
</tr>
<tr>
<td>R0108</td>
<td>35</td>
<td>3</td>
<td>Never</td>
</tr>
<tr>
<td></td>
<td>27/8</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>R0120</td>
<td>97</td>
<td>&gt;100</td>
<td>Never</td>
</tr>
<tr>
<td>R0156</td>
<td>88</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>F0106</td>
<td>59</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>F0112</td>
<td>64</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>F0113</td>
<td>49</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>F0114</td>
<td>75</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>F0117</td>
<td>57</td>
<td>4</td>
<td>Never</td>
</tr>
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<td>F0119</td>
<td>67</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>F0120</td>
<td>61</td>
<td>8</td>
<td>20</td>
</tr>
</tbody>
</table>

R, roofers; F, foundry workers.  
Repeated on a different day.  
1:10 dilution of DNA.  
1:40 dilution of DNA.  
DNA extracted from second aliquot of blood.

Elkart, IN; 1:1000, 100 μl/well) to the wells and incubated at 37°C for another hr. The wells were then washed 3 times with PBS-Tween and twice with 0.1 M diethanolamine buffer, pH 9.0, and then treated with substrate (0.1 mg p-nitrophenyl phosphate, 100 μl/well in 1 M diethanolamine buffer for ELISA; 20 pg/well [3H]AMP for USERIA). The end point of the antigen-antibody reaction was determined by alkaline phosphatase activity which, in turn, was quantitated either by spectrophotometric determination of absorbance at 405 nm (for ELISA) or by chromatographic separation of [3H]adenosine and subsequent counting of radioactivity (for USERIA) (14).

Percentage inhibition of the test samples were determined from the standard curves. All tests and assays were done in duplicates, and the values between duplicates varied less than 10%. The assays were performed blindly by coding the test samples and controls. Control DNA was obtained from 9 volunteer laboratory personnel and also from 2 aliquots of the human B-lymphocyte cell line (RPMI 4265) and commercial calf thymus DNA.
not surprising that BPDE:DNA adducts have been identified in them. This positive antigenicity suggests the presence of BPDE:DNA adducts (18, 25). Our results indicate that further investigations are worthwhile. For example, studies are necessary to determine the persistence of the adducts or efficiency of removal of the adducts and the quantitative relationship between BPDE:DNA adducts in WBC and “target” organs, e.g., lung. Although their relevance in terms of carcinogenicity in humans remains to be determined, this biochemical epidemiological approach provides an opportunity to test for the presence of carcinogen:DNA adducts in exposed individuals.

The central dogma in chemical carcinogenesis can be summarized as follows: (a) the organism is exposed to environmental chemicals, most of which are procarcinogens that need metabolic activation; (b) the metabolically active ultimate carcinogen reacts with cellular macromolecules, such as DNA, RNA, or proteins; and (c) the lesions in the DNA may persist or may be repaired. Although the population is exposed to various environmental carcinogens, only a fraction thereof develops cancer. This difference may be partly explained by the wide interindividual variation in the ability of the organisms to handle each of the above steps, i.e., exposure, metabolism, adduct formation, and DNA repair (5, 12, 13, 29). It thus seems that the level of carcinogen:DNA adduct in any particular tissue at a given time is the net result of exposure, activation, and repair. Therefore, monitoring of carcinogen:DNA adducts at different times following known exposure may provide insight into the efficiency of metabolic activation. Likewise, persistence of the adducts and the efficiency of DNA repair may be estimated by monitoring the adduct level following cessation of the exposure. Prospective population studies are needed to investigate the relationship of adduct levels with eventual risk of cancer. Nevertheless, identification of adducts in exposed humans may serve as a monitoring device for those who are at putatively high risk.

Having identified 2 occupational groups with high exposure to BP, we have detected BPDE:DNA adducts in their peripheral blood. Interestingly, both of the 2 cigarette smoking volunteers (out of a total of 9) were also positive for BPDE:DNA adducts. Thus, the assay appears to be a very sensitive indicator of BP exposure. Caution must, however, be exercised, since BP is a ubiquitous carcinogen. It might not be too surprising to find low levels of BPDE:DNA adducts in people of certain dietary, social, or personal habits. For instance, besides tobacco smoke, BP has been found in various oils, margarine, butter, fats (up to 62 μg/kg) (2); fruits, vegetables, and cereals (up to 48.1 μg/kg) (15); periphral blood and testing by USERIA is relatively simple and, since the procedure of DNA isolation from human cultured lymphocytes and by human liver microsomes. Pharmacology, 17: 241—248, 1976.

ACKNOWLEDGMENTS

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