Detection of Polycyclic Aromatic Hydrocarbon-DNA Adducts in White Blood Cells of Foundry Workers

F. P. Perera, K. Hemminki, T. L. Young, D. Brenner, G. Kelly, and R. M. Santella


ABSTRACT

Iron foundry workers, exposed to high levels of polycyclic aromatic hydrocarbons (PAHs), silica, and metal fumes and dusts, are at elevated risk of lung cancer. Benzo(a)pyrene and a number of structurally related PAHs are metabolically activated to diol epoxides (e.g., 7,8,8a-trihydroxy-9a,10e-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene) which are mutagenic, carcinogenic in experimental animals, and form covalent adducts with DNA. The levels of these adducts were measured in an enzyme-linked immunosorbent assay using a polyclonal anti-benzo(a)pyrene diol epoxide-I-DNA antibody which cross-reacts with DNA modified by diol epoxides of structurally related PAHs. DNA was analyzed from peripheral blood cells of 35 Finnish foundry workers and 10 controls. Workers were classified as having low (<0.05 ng/mg), medium (0.05-0.2 ng/mg), or high (>0.2 ng/mg) exposure to benzo(a)pyrene (as an indicator of PAH). When adjustment was made for cigarette smoking and time since vacation, benzo(a)pyrene exposure was significantly related to adduct levels (P = 0.0001). Each of the three exposure groups had significantly elevated adduct levels compared to controls. Among the exposed workers, the low group differed significantly from the high and medium categories. This study supports the usefulness of monitoring adduct formation in a population occupationally exposed to carcinogens.

INTRODUCTION

Cancer epidemiology can be greatly enhanced by methods to quantify the biologically effective dose of carcinogens (the amount of activated carcinogen that has interacted with critical cellular targets) (1-3). This information can be valuable in understanding "dose-response" relationships and in flagging potentially elevated cancer risks. Carcinogen-DNA adducts are viewed as a useful and relevant marker of biologically effective dose because of evidence that covalent binding to DNA is a critical event at one or more stages of tumorigenesis (4-6), and that carcinogenic potency of a series of PAHs correlates with their ability to form specific DNA adducts (7, 8). However, experimental evidence suggests that PAH-DNA adducts are a necessary but not sufficient event in tumor formation (9).

Incomplete combustion of organic materials, including fossil fuels, is the major source of PAHs, such as BP, generally used as a representative indicator of total PAH concentrations, BA, benzo(â)fluoranthene, and chrysene. For example, BP is a ubiquitous pollutant encountered in the workplace, urban air, drinking water, and the food supply (10, 11). It is a constituent of mainstream (20-40 ng/cigarette) and sidestream cigarette smoke (68-136 ng/cigarette) (12).

As a constituent of combustion mixtures, BP is linked to increased risk of lung cancer in smokers, nonsmokers (via passive smoking) (12-14), and in occupational groups such as coke oven workers (15), and foundry workers (16). Thus foundry workers constitute a model population for purposes of validating PAH-DNA adducts as a marker of biologically effective dose.

Prior studies have detected PAH-DNA adducts in lung tissue from lung cancer patients (17), in placental tissue (18), in peripheral WBC DNA from smokers and nonsmokers (19), in roofer's (20), coke oven workers (21) and in foundry workers (20). Antibodies elicited against BPDE-I-DNA were used to establish a sensitive ELISA (22). They were later shown to cross-react with DNA modified by diol epoxides of other PAHs which form adducts with stereochemistry similar to BPDE-I-DNA, such as BA (20-10-fold lower affinity than BP) and chrysene (with a higher affinity) (23, 24). These PAHs are found in the same sources as BP, in fairly similar concentrations (e.g., BA, 40-70 ng/cigarette; chrysene, 40-60 ng/cigarette) (12). Levels of BP, BA, and chrysene are also, reportedly, comparable in foundry air (16). Thus positive reaction with the antibody may indicate the presence of multiple PAH-DNA adducts.

MATERIALS AND METHODS

Forty-five individuals were enrolled in the study between August 1985 and May 1986. Thirty-five were employed at an iron foundry in Finland, while the remaining 10 were unexposed controls referred to the Institute of Occupational Health for possible occupational disease. The foundry workers and controls were similar in terms of age and sex distribution. Workers were classified with regard to current smoking status (average number of cigarettes smoked per day). Among the foundry workers, 12 individuals were current nonsmokers, while 23 (66%) smoked an average of 21 ± 5 (SD) cigarettes/day. Among the controls, there were 6 nonsmokers and 4 smokers (40%), with an average of 13 ± 3 (SD) cigarettes/day. Thus, smoking levels among smokers in each of the three foundry worker exposure categories were comparable; however, smoking controls had a lower average cigarette consumption.

The foundry workers were stratified according to current job category. Each job category was assigned an exposure rank by two industrial hygienists familiar with the workplace. Their assessment was based on industrial hygiene surveys (1979-1980) involving extended sampling times, industrial hygiene surveys (1979-1980) involving extended sampling times, and more than 100 workplace area measurements of PAHs, including BP (µg/m³). As industrial hygiene and production processes have not changed essentially in the past 10 years, these surveys represent a fairly accurate estimate of current exposures. Workers were assigned to job and exposure categories based on their current job within the plant; this classification was considered representative of exposure during the past 5-10 years. Jobs involving 10-h TWAs greater than 0.2 µg/m³ were classified as "high exposure," those with TWAs exposures between 0.05 and 0.2 µg/m³ were characterized as "medium," while those with TWAs exposure of less than 0.05 µg/m³ were placed in the "low" category. Peak BP levels of 2-3 µg/m³ were measured in casting and shakeout areas of the foundry.

All workers in this plant take their annual month-long vacation
RESULTS

As part of an interlaboratory comparison of antibodies, assays, and standards,4 we have used several BPDE-I-DNA samples modified in vitro to validate adduct recovery with the fluorescent ELISA. Previously, we have routinely used a highly modified DNA sample (5 adducts/10^10 nucleotides, 15 pmol/µg) to generate standard curves. Modification in this sample is expressed in terms of fmol BPDE-I-deoxyguanosine adduct which would cause similar inhibition per µg DNA.

Calf thymus DNA was modified to a level of 5 adducts/10^9 nucleotides (15 pmol/µg) in vitro with BPDE-I as described previously (26). Modification level was determined from the absorbance at 347 nm (<20% inhibition were considered positive. For analytic purposes, results were assigned a value of 0.03 fmol/µg, an amount midway between the lowest positive fmol/µg concentration and zero. Results are the mean of a single assay with triplicate wells. As mentioned above, measured antigenicity may result from multiple diol epoxide adducts; however, because the standard curve is constructed using BP-DNA adducts, modification level is expressed in terms of fmol BPDE-I-deoxyguanosine adduct which would cause similar inhibition per µg DNA. As part of an interlaboratory comparison of antibodies, assays, and standards,4 we have used several BPDE-I-DNA samples modified in vitro to validate adduct recovery with the fluorescent ELISA. Previously, we have routinely used a highly modified DNA sample (5 adducts/10^10 nucleotides, 15 pmol/µg) to generate standard curves. Modification in this sample is determined by UV absorbance as discussed in the section above. More recently, we have tested a standard with about a 3400-fold lower modification level, containing only 1.5 adducts/10^9 nucleotides (4.5 fmol adduct/µg DNA). This is close to the lowest modification level measurable with [3H]BPDE-I. The two standards have been run a total of 16 times with an average 50% inhibition for the low modified standard of 45 ± 22 (SD) fmol and with the high modified standard at 4.5 ± 1.5 (SD) fmol. A sample set of standard curves is given in Fig. 1 with 50% inhibition for the highly modified sample at 4.5 fmol adduct/well and for the low at 48 fmol adduct/well. Thus, we have shown that the antibody detects adducts in low modified DNA (1.5 adducts/10^9 nucleotides) with about 10-fold lower efficiency than it can detect them in more highly modified DNA (5 adducts/10^9 nucleotides). It is therefore more appropriate to use a standard with low modification for our human samples. This difference between high and low modified standards was discovered after the human samples had been assayed, using a highly modified standard. As a result, values for adducts detected in the biological samples given in Table 1 have been “corrected” to better reflect the actual modification levels by multiplication of fluorescent ELISA values by a factor of 10. Currently, we are routinely using a low modified standard for all human samples to eliminate the need for this correction.

As shown in Table 1, the percentage of individuals with positive samples increased with exposure (controls, 20%; low exposure, 72%; medium and high exposure, 100%). A dose response was seen in that adduct levels increased with increasing exposure (Table 1). Analysis of covariance was used to compare groups with respect to the response variable (adducts) in the presence of possible confounding variables (27). One of the assumptions underlying the analysis of covariance method is that the variability in the response variable is the same for all four groups. Examination of the standard deviations of the adduct levels in Table 1 shows that this assumption is not satisfied in that the standard deviation increases as average adduct level increases. A transformation of the data was therefore necessary to stabilize the variances (27). Analysis showed that between-group variation in exposure was significantly related to the variation in adduct (P = 0.0001). Smoking status

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Fig. 1. Competitive inhibition of antibody binding to plates coated with BPDE-I-DNA modified to a level of 15 pmol/µg. The competitors were BPDE-I-DNA modified to a level of 15 fmol/µg (1) or 4.5 fmol/µg (3). For additional details, see "Materials and Methods."

<table>
<thead>
<tr>
<th>Exposure group</th>
<th>Control</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of individuals</td>
<td>10</td>
<td>18</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>Mean age (yr)</td>
<td>43.2</td>
<td>41.6</td>
<td>42.2</td>
<td>46.0</td>
</tr>
<tr>
<td>(SD)</td>
<td>(10.0)</td>
<td>(10.1)</td>
<td>(10.3)</td>
<td>(08.0)</td>
</tr>
<tr>
<td>Mean no. cigarettes/day</td>
<td>5.0</td>
<td>14.8</td>
<td>13.7</td>
<td>10.0</td>
</tr>
<tr>
<td>(SD)</td>
<td>(6.7)</td>
<td>(12.3)</td>
<td>(10.3)</td>
<td>(11.5)</td>
</tr>
<tr>
<td>No. positive</td>
<td>2</td>
<td>13</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>% positive</td>
<td>20</td>
<td>72</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Mean adduct level (fmol/µg)</td>
<td>0.066</td>
<td>0.24</td>
<td>0.62</td>
<td>1.5</td>
</tr>
<tr>
<td>(SE)</td>
<td>(0.028)</td>
<td>(0.057)</td>
<td>(0.15)</td>
<td>(0.55)</td>
</tr>
<tr>
<td>Range</td>
<td>0.030-0.3</td>
<td>0.030-0.86</td>
<td>0.10-2.0</td>
<td>0.80-2.8</td>
</tr>
<tr>
<td>Mean adducts/10^9 nucleotides</td>
<td>0.22</td>
<td>0.80</td>
<td>2.1</td>
<td>5.0</td>
</tr>
<tr>
<td>Exposure level (µg/m³ BP)</td>
<td>&lt;0.05</td>
<td>0.05-0.2</td>
<td>&gt;0.2</td>
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</tr>
</tbody>
</table>
and time since vacation were included as covariates since prior studies have indicated the potential importance of smoking (19) and since the exposure groups differed significantly (by the F test) in terms of the average length of time elapsed between the annual 1-month vacation and sample collection. The effects of smoking (number of cigarettes smoked/day) on adduct levels were not statistically significant ($P = 0.20$), nor was that of time since vacation ($P = 0.34$). Sex and age distribution was comparable between groups and were not included as covariates.

Individual comparisons of average log adduct levels (unadjusted for the covariates) were made between the groups, using a 2-sample $t$ test. The $P$ values of the $t$ test were modified by using the Bonferroni criterion to adjust for the fact that multiple pairwise comparisons were made (28). As shown in Table 2, the three exposed groups all had statistically significant higher average log adduct levels (and de facto higher average adduct levels) than the control group. Among the exposed groups, the low group differed significantly from the high and medium groups ($P < 0.05$). The difference between the high and medium groups was not significant, but the high group had the highest average adduct level. It is clear that the groups exposed to PAH have higher adduct levels than the control group when age, sex, smoking history, and time since vacation are taken into account. Finally, the mean adduct level of samples collected from 4 workers immediately after 4 weeks of vacation was $0.060 \pm 0.028$ (SE) compared to the mean level of adducts found after 6 weeks of foundry work [$0.53 \pm 0.46$ (SE)].

**DISCUSSION**

In this most detailed occupational study of DNA adducts carried out thus far, we have utilized a highly sensitive immunoassay to evaluate the relationship between workplace air levels of BP as an indicator of PAH concentrations and extent of formation of levels of DNA adducts by BPDE I and related PAH-diol epoxides. The immunoassay is dependent on modification level of the DNA in that adducts are seen more efficiently at higher modification levels than at low levels. A similar difference in antibody reactivity with modification level has also been seen by others (29). Since the human samples have a low modification level, it is more appropriate to use a low modified standard. Even with this standard, however, there are difficulties in obtaining absolute values for modification levels. The measured antigenicity of a sample results from antibody binding with variable affinity to the various PAH trans-dial epoxide adducts which might be present. While we have determined the ratio between binding to high and low modified BPDE-I-DNA, we do not know the comparable ratios for DNA modified by trans-diol epoxides of BA, chrysene, etc. Thus, it should be remembered that this ELISA provides a general measure of PAH trans-diol epoxide DNA adduct levels.

In this population with relatively well characterized exposure and known current smoking status a clear dose response was seen: adducts were significantly related to BP exposure and were not significantly influenced by current cigarette smoking or by time since vacation.

In a prior study of foundry workers, the range of adducts in the 7 of 20 samples found positive for BPDE-DNA antigenicity by the competitive ultrasensitive enzymatic radioimmunoassay was 0.06-0.4 fmol/μg. Information on the remaining 13 samples was not provided, nor were workers classified as to exposure (20). Somewhat higher levels of adducts were seen in coke oven workers (30).

The study is limited by the relatively small sample size, by the fact that personal monitoring data, past smoking history, and data on other environmental sources of PAH (diet, passive smoking, ambient air, etc.) were not obtained. Moreover, we were unable to collect serial blood samples from each individual immediately after his/her return from the annual month-long vacation and again following 6 weeks of work. Our results for 4 individuals showing a significant difference in adduct levels in these paired samples suggest, however, that there is a rapid acquisition of adducts in WBC. This is consistent with the short lifetimes of a significant fraction of WBC (31), but may also indicate rapid repair of a proportion of adducts when exposure is discontinued.

In conclusion, this study in an occupationally exposed population confirms the usefulness of the ELISA as a carcinogen dosimeter and indicator of potential risk of cancer.

**ACKNOWLEDGMENTS**

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**REFERENCES**

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