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., 7,8,9,10-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 µg/m³), medium (0.05–0.2 µg/m³), or high (>0.2 µg/m³) exposure to benzo[a]pyrene (as an indicator of PAH). When adjusted 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(k)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).

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 (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 stereochromy similar to BPDE-I-DNA, such as BA (with a 5- to 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 ± 6 (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 and more than 100 workplace area measurements of PAHs, including BP (µg/m³). As industrial hygiene and production processes have 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, since individuals in this plant tend to remain in the same job and are generally long-term employees. Jobs involving 8-h TWA greater than 0.2 µg/m³ were classified as “high exposure,” those with TWA exposures between 0.05 and 0.2 µg/m³ were characterized as “medium,” while those with TWA 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.
simultaneously, during the month of July. Thus it was possible to
calculate for each worker the time elapsed between vacation and blood
sample collection. This variable was included in the analysis. In addi-
tion, we studied four workers from whom blood samples were collected
immediately after their return from vacation and again, after 6 weeks
of exposure.

All subjects gave one or more peripheral blood samples. Blood
samples (30-50 ml) were collected in heparinized plastic tubes, coded,
and centrifuged; buffy coat cells, RBC, and plasma were collected and
frozen at —70°C. DNA was isolated in Finland by standard phenol and
RNeasy treatment (25). Ethanol-precipitated samples were dried and
shipped to Columbia University for analysis. Coded samples were
assayed by competitive ELISA, essentially as described previously (17),
with fluorescence detection. Briefly, 96-microwell black plates
(MicroFLUOR “B”; Dynatech Laboratories, Alexandria, VA) were
coated with 0.5 ng BPDE-I-DNA (5 adducts/10^9 nucleotides or 15
pmol/µg). A previously characterized rabbit polyclonal antibody (22)
was used at a 1:8,000 dilution. A standard curve was constructed by
mixing 50 µl diluted antibody with in vitro modified BPDE-I-DNA in
carrier nonmodified calf thymus DNA such that 50 µl contained from
0.25-25 fmol BPDE-I-deoxyguanosine adduct in 50 µg DNA. All
human samples were also assayed at 50 µg DNA/well after sonication
and denaturation by boiling for 3 min and cooling on ice. A conjugate
of goat anti-rabbit IgG-alkaline phosphatase (Sigma Chemical Co., St.
Louis, MO) was used at 1:400 dilution. The substrate, 4-methylumbel-
liferyl phosphate (100 µl, 50 µg/ml 0.1 M diethanolamine, pH 9.6)
becomes fluorescent after phosphate removal. Fluorescence was read
on a Microfluor reader (Dynatech Laboratories). Samples with greater
than 20% inhibition were considered positive. For analytic purposes,
samples with assays showing less than 20% inhibition were classified as
“nondetectable” and each was 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 inhibi-
tion per µg DNA.

Calf thymus DNA was modified to a level of 5 adducts/10^9 nucleo-
tides (15 pmol/µg) in vitro with BPDE-I as described previously (26).
Modification level was determined from the absorbance at 347 nm (ε
= 29,000). A sample of calf thymus DNA modified to a level of 1.5
adducts/10^6 nucleotides (4.5 fmol adduct/µg) with [^H]BPDE-I, was kindly
provided by Ainsley Weston of NIH, Bethesda, MD. Modification level
was determined from the specific activity of the DNA.

RESULTS

As part of an interlaboratory comparison of antibodies, as-
says, and standards, we have used several BPDE-I-DNA sam-
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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 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 ± 0.028 (SE) compared to the mean level of adducts found after 6 weeks of foundry work [0.53 ± 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, it is more appropriate to use a low modified antigen to measure antigenicity of a sample results from antibody binding with variable affinity to the various PAH trans-diolepoxide 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-diolepoxide of BA, chrysene, etc. Thus, it should be remembered that this ELISA provides a general measure of PAH trans-diolepoxide 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**


**Table 2 Pairwise comparisons of log fmoles/µg adduct levels among the four exposure groups**

<table>
<thead>
<tr>
<th>Comparison groups</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control:low</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Control:medium</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control:high</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Low:medium</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Low:high</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Medium:high</td>
<td>NS*</td>
</tr>
</tbody>
</table>

* Adjusted by Bonferroni criteria.
* NS, not significant.
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