Polycyclic Aromatic Hydrocarbon-DNA Adducts in White Blood Cells and Urinary 1-Hydroxypyrene in Foundry Workers

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Abstract
In an ongoing comprehensive evaluation of biological markers, workers in or near an iron foundry with varying exposure to polycyclic aromatic hydrocarbons (PAH) were analyzed for molecular response to this exposure. Exposure to benzo(a)pyrene, determined by personal monitors worn by the workers (2 to 60 ng/m²), was considerably lower than in a previous study at this foundry (<50 to 200 ng/m²) (F. P. Perera et al., Cancer Res., 48: 2288-2291, 1988). Two biomarkers, 1-hydroxypyrene in urine measured by high-performance liquid chromatography with fluorescence detection (a measure of internal dose) and PAH-DNA adducts in WBC measured by immunoassay (a measure of biologically effective dose) were assessed to demonstrate their relationship to the lowest exposures yet analyzed in foundry workers. In addition, these markers were analyzed for dose response and interindividual variability. Cigarette smoking, but not age or charbroiled food, influenced the level of 1-hydroxypyrene but not PAH-DNA adducts. When workers were classified into three exposure categories (low, medium, and high), mean 1-hydroxypyrene levels were 2.7, 1.8, and 3.6 µmol/mol creatinine, respectively. Comparisons by analysis of variance showed a significant difference between the groups after controlling for smoking (P = 0.02), but a trend test using multivariate linear regression analysis was not significant (r = 0.27; P = 0.07). Substantial interindividual variation was demonstrated by the 19- to 20-fold range in the values within each of the three exposure groups. PAH-DNA adducts showed an increasing trend, with exposure from 5.2 to 6.1 to 9.6 adducts/10⁸ nucleotides in the low-, medium-, and high-exposure groups, respectively (P = 0.08). The three exposure groups did not significantly differ from each other by analysis of variance (P = 0.23). There was a 10- to 35-fold range in PAH-DNA adducts within exposure groups, reflecting the interindividual variability in this molecular response to PAH exposure. The correlation (r) between the two markers was 0.15 (P = 0.37). These results indicate that even at relatively low levels of benzo(a)pyrene (approximately 30-fold lower than in the previous study), we continue to observe a dose-response relationship between external exposure and the biologically effective dose of PAH.

Introduction
This study is part of an ongoing comprehensive evaluation of biological markers in iron foundry workers with varying exposures to PAH and at increased risk of lung cancer (1). A major purpose of the research is to investigate interindividual variation in biological response to carcinogenic exposure. Of interest was the relationship between PAH exposure (based on personal monitoring) and levels of two biological markers, 1-hydroxypyrene (an internal dosimeter) and PAH-DNA adducts (as a measure of biologically effective dose). For chemical carcinogens, the biologically effective dose is defined as the amount of carcinogen bound to DNA in the target tissue or, as in this study, a surrogate. Previous studies in this foundry have demonstrated a significant effect of exposure on PAH-DNA adducts in peripheral WBC measured by both immunoassay (2) and ³²P postlabeling (3, 4). Elevated levels of adducts in foundry workers by ELISA have also been reported by others (5). Carcinogen-DNA adducts are considered to be an end point which is mechanistically and biologically relevant to carcinogenesis. Prior studies have also found increased levels of 1-hydroxypyrene in the urine of coke oven workers (6), aluminum plant workers (7), and workers exposed to PAH through the handling of petroleum coke dust (8) and bitumen (9). Measurement of urinary 1-hydroxypyrene, the major metabolite of pyrene, is less invasive than measurement of DNA adducts in blood and may thus be more appropriate for routine workplace monitoring. In addition environmental pyrene levels correlate with total

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1 The abbreviations used are: PAH, polycyclic aromatic hydrocarbon; BP, benzo(a)pyrene; ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography.
PAH ($r = 0.82; P < 0.0001$), indicating that pyrene is a good marker for PAH exposure (7). BP levels also correlate with total PAH (1). We have compared WBC PAH-DNA adducts and urinary excretion of 1-hydroxypyrene in Finnish foundry workers. Personal monitoring of workers indicated that exposure had decreased about 30-fold since our prior studies in this foundry (2).

Materials and Methods

Subjects and Data Collection

The overall cohort under study consists of 48 workers in a Finnish iron foundry aged 19 to 60 years (average, 42 years) and comprising 37 males and 11 females. Fifty-eight % were current smokers, with an average of 24 pack-years of smoking. Their average length of employment in the plant was 13 years (range, 2 to 46 years). All subjects had been employed at the plant for at least 2 years. The group is an ethnically and culturally homogeneous Finnish population. Subjects were interviewed at enrollment using a standardized questionnaire to elicit occupational, diet, smoking, health, and environmental histories. Each individual provided a 30-ml blood sample in heparinized vacutainers which was processed immediately to separate plasma, RBC, and WBC. WBC were sent to the United States for extraction of DNA for adduct analysis. Each worker also donated a urine sample for analysis of 1-hydroxypyrene. All samples were coded before analysis. Due to sample availability and assay requirements data are not available for all subjects in each assay.

Exposure Assessment

Personal monitoring was carried out on the day of sampling or the day before for the whole working day (7 h). Air (approximately 1 m$^3$) was pumped through glass fiber filters. The filters were weighted for total dust content and PAH extracted into dichloromethane. PAHs were analyzed by HPLC using fluorescence detection (10). Stationary samples were collected from 7 points in the foundry and 4 in the control areas on 2 separate days. Dust and PAH analysis was carried out as above. The exposure categories (low, <5; medium, 5–12; high, 12 ng BP/m$^3$) were formed based on personal sampling of BP. In cases when personal data were not available (mainly low exposures), stationary samples from the appropriate work areas were used.

Assays

PAH-DNA Adducts by Competitive ELISA. DNA was isolated from buffy coats by standard phenol/chloroform extractions and RNase treatment. PAH-DNA adducts were measured by a competitive ELISA with fluorescence end point detection essentially as described previously (2). Polyclonal antisera (11) was used at 1:1.6 × 10$^6$ dilution, and the dilution curve was generated with a low modified (±)-7,8,9-9α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene-DNA (1.5 adduct/10$^6$ nucleotides). Alkaline phosphatase-labeled secondary antiserum was used, and the substrate was 4-methylumbelliferyl phosphate. Samples were run in triplicate, and median values were used for determination of percent inhibition. For statistical purposes, those samples with <15% inhibition were considered nondetectable and assigned a value of 1/10$^6$, an amount midway between the lowest positive value and zero. The antiserum recognizes multiple, structurally related PAH diol epoxide-DNA adducts (12, 13), but values are expressed as the amount of (±)-7,8,9-9α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene-DNA which would cause a similar inhibition in the assay.

1-Hydroxypyrene by HPLC. 1-Hydroxypyrene was measured in the urine by HPLC with fluorescence detection essentially as described (10). Urine samples (5 ml) were adjusted to pH 5.0 and incubated overnight with β-glucuronidase/arylsulfatase. Samples were purified by C$_{18}$ reversed-phase liquid chromatography (Sep-Pak C$_{18}$; Waters, Milford, MA). Analysis was carried out by HPLC with fluorescence detection (excitation at 242 nm and emission at 388 nm). Peak heights were used for quantitation.

Statistical Analysis

Biomarker data were log transformed to stabilize the variance and to obtain a more symmetric distribution. However, means and SDs are presented as untransformed values for ease of interpretation. Relationships among all variables of interest (In 1-hydroxypyrene, In PAH-DNA, age, cigarettes per day, and charbroiled meat) were investigated by calculating univariate Pearson’s product moment correlation coefficients. Analysis of variance was used to compare the three exposure groups with respect to biomarker levels. For the regression analysis, low-, medium-, and high-exposure groups were represented by mean BP exposures of 2.0, 6.8, and 19 ng/m$^3$, respectively.

Results

Personal monitoring data indicated that worker exposure in this foundry had decreased dramatically since 1987, when workers were divided into three exposure categories of low (<50), medium (50–200), and high (>200 ng BP/m$^3$) (2). In the present investigation, monitoring data indicated the highest exposure was 60 ng BP/m$^3$. Workers were thus divided into low (<5), medium (5–12), and high (>12 ng BP/m$^3$) exposure categories for analysis. Table 1 indicates the number of workers in each category.

<table>
<thead>
<tr>
<th>Exposure level</th>
<th>Low (&lt;5 ng BP/m$^3$)</th>
<th>Medium (5–12 ng BP/m$^3$)</th>
<th>High (&gt;12 ng BP/m$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>14</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>Mean*</td>
<td>2.7 ± 2.2</td>
<td>1.8 ± 1.2</td>
<td>1.6 ± 2.5</td>
</tr>
<tr>
<td>Range*</td>
<td>0.3 – 6.1</td>
<td>0.1 – 4.2</td>
<td>0.5 – 9.7</td>
</tr>
<tr>
<td>Mean (adjusted)*</td>
<td>2.3 ± 1.9</td>
<td>1.1 ± 0.7</td>
<td>2.5 ± 1.7</td>
</tr>
</tbody>
</table>

a ng/ml creatinine

Table 1 Summary of assay results

Adducts/lO$^6$ nucleotides.
Mean urinary levels of 1-hydroxypyrene in the low-, medium-, and high-exposure groups were 2.7, 1.8, and 3.6 μmol/mol creatinine (Table 1; Fig. 1). In contrast to cigarette smoking, age and charbroiled meat did not influence levels of 1-hydroxypyrene. Since 1-hydroxypyrene is a measure of recent exposure [half-life of 4.4 h (14) or 6–35 h (6)], values were adjusted for recent smoking (cigarettes per day, Table 1). Comparisons between the BP exposure groups by analysis of variance showed a significant difference after adjusting for smoking (P = 0.02). By multivariate linear regression, 1-hydroxypyrene was associated with BP exposure, with cigarette smoking included in the model (r = 0.27; P = 0.07). Substantial interindividual variation was demonstrated by the 14- to 21-fold range in the values within each of the three exposure groups.

By linear regression mean PAH-DNA adducts in peripheral WBC, measured by competitive ELISA, increased with exposure from 5.2 to 6.1 to 9.6 adducts/10⁸ nucleotides (r = 0.28; P = 0.08) (Fig. 1). The three exposure groups did not significantly differ from each other by analysis of variance (P = 0.23). Adducts were somewhat (although not significantly) higher in smokers (7.54/10⁸; n = 27) than in nonsmokers (5.16/10⁸; n = 11; P = 0.38). Age and charbroiled meat were not significantly associated with PAH-DNA adduct levels. There was a 10-, 14-, and 35-fold range in PAH-DNA adduct values in the low-, medium-, and high-exposure groups, respectively, reflecting the interindividual variability in this molecular response to PAH exposure. The correlation (r) between urinary 1-hydroxypyrene and PAH-DNA adducts was 0.15 (P = 0.37). It is of interest that the subject (high-exposure group) who had the highest value for PAH-DNA adducts (35/10⁸) was also the highest in terms of 1-hydroxypyrene in urine (9.7 μmol/mol).

Discussion

These studies demonstrate increased levels of urinary 1-hydroxypyrene and PAH-DNA adducts in WBC resulting from occupational exposure to PAH. Workers in the highest exposure group in the present study (>12 ng/m³) had exposures similar to those in the lowest exposure group in our prior study at this foundry (<50 ng/m³) (2). PAH-DNA adduct levels in these 2 groups were similar (9.6/10⁸ in this study and 8.0/10⁸ in the prior study). A small but nonsignificant increase in adduct levels was detected with cigarette smoking exposure, possibly due to the small number of smokers. Prior studies by both ELISA and ³²P postlabeling have produced conflicting results on adducts in WBC of smokers (reviewed in Ref. 15). More recently, we have demonstrated that ³²P postlabeling (16) and ELISA (17) can detect increased adducts in cigarette smokers when the lymphocyte plus monocyte fraction is assayed. We have also recently shown that PAH-DNA adducts in total WBC are increased in heavy smokers with high levels of cotinine.

Increases in urinary excretion of 1-hydroxypyrene in workers occupationally exposed to PAHs have been ob-

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*Mooney et al., submitted for publication.*
PAH-DNA Adducts and 1-Hydroxypyrene in Foundry Workers

served in several studies (6, 7, 18–20). In a prior study of coke oven workers with substantially higher (50–130-fold) exposure to BP (0.1–7.8 µg/m³) and pyrene (0.6–23.6 µg/m³), concentrations of 1-hydroxypyrene ranged from nondetectable to 11.2 µmol/mol in workers, with means of 1.78 to 3.37 µmol/mol at the end of shift on different days (6, 21). Elevated levels of PAH-DNA adducts measured by ELISA were also seen in these coke oven workers (5.1/10⁸) compared to controls (2.7/10⁸) (21); but no significant correlations were found between adducts in blood and air concentrations of total PAH or urinary 1-hydroxypyrene. However, a study of aluminum plant workers did find a correlation between urinary 1-hydroxypyrene and total PAH (r = 0.62; P = 0.006) (7).

Dietary sources of exposure to PAH have also been demonstrated to influence urinary excretion of 1-hydroxypyrene (14). A 100- to 250-fold increase in dietary BP paralleled a 4- to 12-fold increase in urinary 1-hydroxypyrene excretion. A similar type of voluntary feeding study has also suggested that pheripheral WBC PAH-DNA adducts measured by ELISA increase after ingestion of charcoal-broiled beef (22).

The three methods used here monitor exposure to PAH over different time scales. The personal monitors measured exposure over the 7-h work period during which they were worn. This measure of daily exposure may vary dramatically from day to day. With a half-life of 4.4–36 h (6, 14), urinary 1-hydroxypyrene is also a measure of recent exposure. The time scale of exposure detectable by measurement of DNA adducts in WBC is less clear. While many cells are short-lived, a certain fraction of lymphocytes are long-lived and may contain persistent adducts resistant to DNA repair. Repeat samples from nine foundry workers after a 1-month vacation and again after 6 weeks of work indicated adduct levels were lower after the vacation (3.9/10⁸) than at work (30.8/10⁸). However, levels were still elevated after the 1-month vacation compared to controls (2.2/10⁸) (21).

The present study, involving exposures 1–2 orders of magnitude lower than either our own prior study of foundry workers or in the coke oven worker study (21), has demonstrated a dose-response relationship between external exposure and both internal and biologically effective dose of PAH. The failure to see a significant correlation between 1-hydroxypyrene and adducts is probably due to the differing half-lives and biology of these biomarkers and the fact that they are variously reflecting current (1-hydroxypyrene) and past exposures (PAH-DNA). These results argue for the use of a combination of biomarkers and industrial hygiene in further molecular epidemiological validation studies.

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References

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