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DNA Adducts As a Measure of Lung Cancer Risk in Humans Exposed to Polycyclic Aromatic Hydrocarbons

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Workers in the coking, foundry, and aluminum industry can be exposed to high concentrations of polycyclic aromatic hydrocarbons (PAHs) and are at increased risk for lung cancer, as are cigarette smokers. In recent years several studies on workers in the foundry and coking industries have been reported. In these studies, white blood cell (WBC) DNA was used for analysis of PAH–DNA adducts. Theoretically, DNA adduct formation is a more relevant biological parameter for assessing exposure risk than PAH in the work atmosphere, or the amount of a metabolite in the urine, because adduct levels reflect that part of the dose that escapes detoxification and binds to DNA. We analyzed WBC DNA from coke-oven workers and from workers in an aluminum production plant and demonstrated the presence of PAH–DNA adducts. Forty-seven percent of the coke-oven workers had detectable levels of PAH–DNA adducts in their WBC compared with 27% of the controls ($p < 0.05$), measured with ELISA. In both groups, smokers had significantly higher levels of PAH–DNA adducts than did nonsmokers. In the aluminum workers, no PAH–DNA adducts were detected by ELISA, although the benzo[*a*]pyrene concentrations in the work atmosphere were comparable to those of the coke-oven workers. The more sensitive ^{32}P-postlabeling assay showed the presence of PAH–DNA adducts in 91% of the aluminum workers. There was no correlation of WBC adduct levels with the concentration of PAH in the work atmosphere. Recently we showed that total PAH–DNA adduct levels in WBC from lung cancer patients were much higher than those generally found in healthy smokers. These increased adduct levels may indicate a subpopulation of smokers with increased risk for lung cancer, resulting from a genetic predisposition in this group of persons. Because WBCs are not the target cells for exposure-related cancer, the relationship between PAH–DNA adducts in the lung and in WBCs remains to be established.

Introduction

A large number of chemical carcinogens, such as polycyclic aromatic hydrocarbons (PAHs), react covalently with DNA and may thereby initiate the multistage process leading to malignancy (*1*). These DNA adducts may serve as quantitative indicators of biologically effective doses of specific carcinogens. Advances in analytical methods during the last decade make possible the sensitive measurement of covalent adducts of carcinogens with DNA in humans (*2*). Methods of PAH–DNA adduct analysis involve immunochemical assays (*3,4*), synchronous fluorescence spectroscopy (SFS)(*5*), and postlabeling of adducted deoxyribonucleotides with [^{32}P]phosphate (*6,7*). The ^{32}P-postlabeling analysis is the most sensitive method, and the high specific radioactivity of [^{32}P]ATP used in this procedure allows detection of 1 adduct in 10^9 bases (*7*). However, resolution of individual PAH–DNA adducts from complex mixtures by TLC is often difficult, and several methods have been developed to improve the resolution of the ^{32}P-labeled adducted nucleotides. HPLC seems to be the most promising approach to optimize the detection of multiple adducts in one DNA sample (*8,9*). In addition to better separation of adducted nucleotides, the HPLC method also facilitates the identification of individual DNA adducts by mass spectrometry.

Immunochemical methods, i.e., immunoassays of adducted DNA or nucleotides and immunocytochemistry of tissues and cells, employ antibodies that are raised against carcinogen-modified DNA or nucleosides or nucleotides (*3,4*). Immunoassays are usually performed as competitive ELISA (enzyme-linked immunosorbent assay). The ^{32}P-postlabeling technique, and in certain cases the ELISA, are sufficiently sensitive to

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detect DNA adducts at the levels encountered in humans. A number of studies have appeared in recent years in which these methods have been used to determine DNA adducts in humans occupationally exposed to PAHs. Some results of our own studies are briefly summarized here and are compared with data reported by other investigators.

Occupational PAH Exposure and DNA Adducts

Air near coke ovens, in iron foundries, and in aluminum production plants contains a number of carcinogenic PAHs (*10*). Epidemiological studies from several countries have shown an increased risk of lung cancer among workers in these industries and the risk appears to correlate with exposure to PAH (*10*). Several laboratories have used the ^{32}P-postlabeling technique and/or immunoassays to determine the biologically effective dose of PAH in humans occupationally exposed to PAHs.

Coke Ovens

In an extensive occupational study on coke-oven workers, we have tested the applicability of the immunologic detection of benzo[*a*]pyrene (BaP)–DNA adducts (*11*). A typical PAH profile of the personal air samples is illustrated in Figure 1*A*, which shows that fluoranthene, benz[*a*]anthracene, and benz(*bjk*)fluoranthene are the most abundant PAHs. Although large fluctuations in absolute concentration were noted over periods of several days, the PAH profile was fairly constant, and BaP can be an appropriate indicator for PAH exposure from the coke ovens. The levels of DNA adducts in WBCs from coke-oven battery workers ranged up to 20.4 adducts/10^8 nucleotides and were significantly increased compared with nonbattery workers ($p < 0.05$) (Fig. 2). No significant correlations were found between PAH–DNA adducts in blood and the air concentrations of total PAHs, BaP separately, or those PAHs that are known to form diol epoxides, but a dose–response relationship was seen between hours exposed and the level of PAH–DNA adducts (r=0.47; p=0.005). Smoking had a significant effect on the PAH–DNA adduct levels; in all groups examined smokers had consistently higher levels of PAH–DNA adducts than did nonsmokers. Our data agree fairly well with those from other studies on coke ovens and iron foundries (Table 1). The higher adduct levels found by other investigators may be the result of different worker exposures to PAH and differences in PAH profiles in the various factories. Comparison of data from different laboratories, however, should be done with caution, for reasons discussed below.

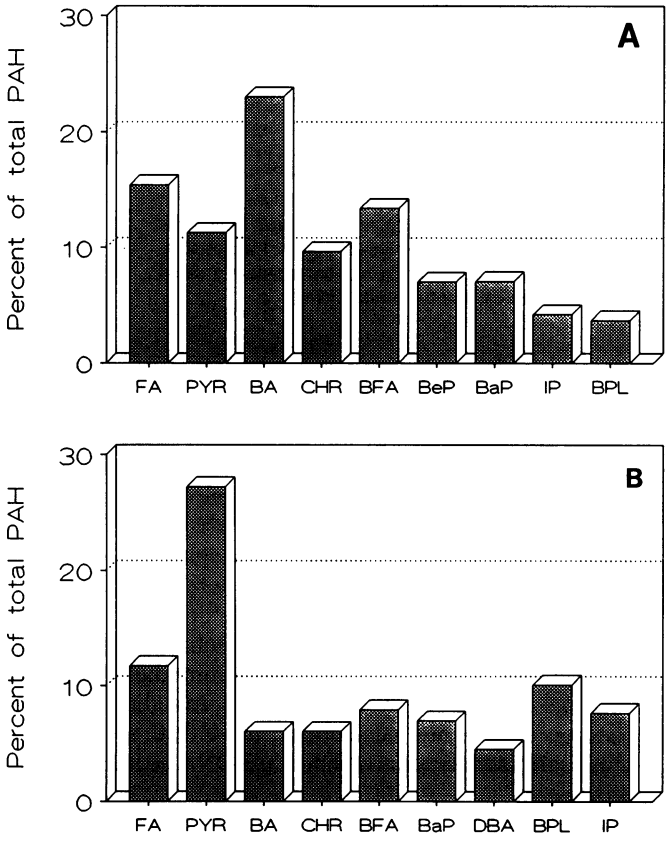

FIGURE 1. Mean PAH profile obtained by personal sampling of (*A*) coke-oven workers and (*B*) workers in an aluminum production plant. The main components found are: FA, fluoranthene; PYR, pyrene; BA, benz[*a*]anthracene; CHR, chrysene; BFA, benzo[*bjk*]fluoranthene; BeP, benzo[*e*]pyrene; BaP, benzo[*a*]pyrene; DBA, dibenz[*a,h*]anthracene; IP, indeno[1,2,3-*cd*]pyrene; BPL, benzo[*ghi*]perylene.

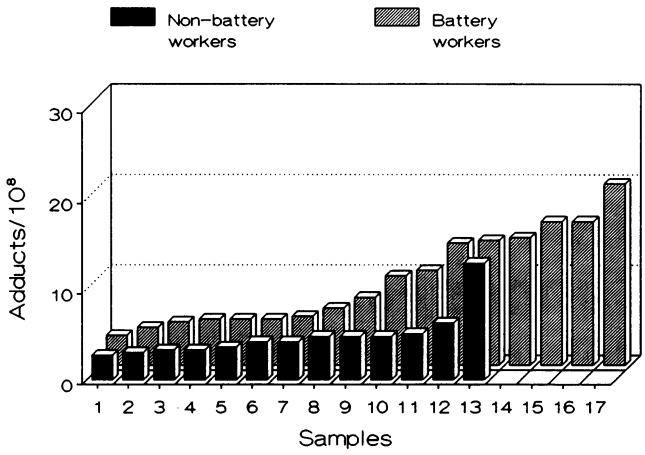

FIGURE 2. White blood cell DNA adducts of individual coke-oven workers involved in battery (shaded bars) and nonbattery (black bars) operations.

Aluminum Production

Relatively few studies have been reported on PAH–DNA adduct levels in workers from aluminum production plants (*5,18*). An extensive occupational study in an aluminum production plant is being performed at present in our laboratory. A typical PAH profile of the personal air samples is shown in Figure 1*B*, which shows that in this industry pyrene is the most abundant PAH. The observed difference between PAH profiles of the coke ovens and aluminum production plant emphasizes the importance of obtaining data on the actual PAH levels present in the surrounding air of the workers. Surprisingly, no PAH–DNA adducts in the WBCs of these workers could be detected by ELISA with our anti-benzo[*a*]pyrene diolepoxide (BPDE)–DNA antibody, although the absolute concentration of BaP did not differ much from that at the coke ovens. Apparently, exposure to

Table 1. DNA adduct levels in workers exposed to PAHs determined by ELISA.

Source	Occupation/process	No. of subjects		Adducts/10^8		Reference
		Assayed	Positive	Mean ± SD	Range	
PBL	Roofers	28	7		1.2–72	Shamsuddin et al., 1985 (*12*)
PBL	Foundry	20	7		1.8–12	Shamsuddin et al., 1985 (*12*)
PBL	Controls	9	2	22.7, 28.2		Shamsuddin et al., 1985 (*12*)
WBS	Foundry/high	4	4		24–48	Perera et al., 1988 (*13*)
WBC	Foundry/medium	13	13		3–60	Perera et al., 1988 (*13*)
WBC	Foundry/low	18	13		0.9–25.8	Perera et al., 1988 (*13*)
WBC	Controls	10	2		0.9–9.0	Perera et al., 1988 (*13*)
WBC	Firefighter	43	15	56.1 ± 48.6		Liou et al., 1989 (*14*)
WBC	Controls	38	13	34.5 ± 13.8		Liou et al., 1989 (*14*)
WBC	Coke oven	31	31	15.3		Hemminki et al., 1990(*15*)
WBC	Controls,urban	15	15	13.0		Hemminki et al., 1990 (*15*)
WBC	Controls,rural	13	13	2.3		Hemminki et al., 1990(*15*)
WBC	Foundry	9	9	30.8 ± 6.9		Hemminki et al., 1990(*16*)
WBC	After vacation	9	9	3.9 ± 1.6		Hemminki et al., 1990(*16*
WBC	Coke oven, CS	16	11	7.3 ± 5.9	1.5–20.4	Van Schooten et al., 1990 (*11*)
WBC	Coke oven, NS	11	6	4.9 ± 5.1	1.5–15.9	Van Schooten et al., 1990 (*11*)
WBC	Controls, CS	28	10	3.0 ± 2.5	1.5–12.9	Van Schooten et al., 1990 (*11*)
WBC	Controls, NS	16	2	1.7 ± 0.7	1.5–4.2	Van Schooten et al., 1990 (*11*)

Abbreviations: PAH, polyaromatic hydrocarbon; ELISA, enzyme-linked immunosorbent assay; PBL, peripheral blood leukocytes; WBC, white blood cells; CS, current smoker; NS, nonsmoker.

Table 2. DNA adduct levels in workers exposed to PAH determined by the ^{32}P-postlabeling.

Source	Occupation/process	No. of subjects		Adducts/10^8 nucleotides		Reference
		Assayed	Positive	Mean ± SD	Range	
WBC	Foundry/high	4	3		0.2–10	Phillips et al., 1988 (*17*)
WBC	Foundry/medium	10	8		0.2–3	Phillips et al., 1988 (*17*)
WBC	Foundry/low	18	4		0.2–1	Phillips et al., 1988 (*17*)
WBC	Controls	9	1		1.9	Phillips et al., 1988 (*17*)
WBC	Coke oven[a]	63	63	11.6/24.5		Hemminki et al., 1990 (*15*)
WBC	Controls/urban[b]	19	19	10.2/21.1		Hemminki et al., 1990 (*15*)
WBC	Controls/rural[c]	15	15	8.4/4.4		Hemminki et al., 1990 (*15*)
PBL	Aluminum/I	25	25	1.48 ± 0.96	0.3–4.1	Schoket et al., 1987 (*18*)
PBL	Aluminum/II	21	21	3.08 ± 1.69	0.4–7.1	Schoket et al., 1987 (*18*)
PBL	Controls	29	29	1.30 ± 0.53	0.2–2.4	Schoket et al., 1987 (*18*)
WBC	Aluminum	15	13	4.6 ± 6.9	0.1–22	Van Schooten et al., unpublished data
WBC	After vacation	14	9	2.2 ± 3.0	0.1–10	Van Schooten et al., unpublished data
WBC	-Day 1[d]	11	10	2.7 ± 4.4	0.1–15	Van Schooten et al., unpublished data
WBC	-Day 3[e]	11	10	9.6 ± 29	0.1–97	Van Schooten et al., unpublished data
WBC	-Day 5[f]	11	8	6.5 ± 11	0.1–30	Van Schooten et al., unpublished data

Abbreviations: PAH, polyaromatic hydrocarbon; WBC, white blood cells; PBL, peripheral blood leukocytes.
[a]Samples from coke-oven workers in Silesia, Poland.
[b]Samples from the local population in Silesia.
[c]Samples from a rural population in Eastern Poland. Adduct levels were determined in two different laboratories (*15*).
[d]Samples taken during the working period.
[f]Samples taken at the end of the working period.

similar levels of individual PAH in the ambient air does not necessarily lead to similar DNA adduct levels in WBCs.

The more sensitive ^{32}P-postlabeling assay showed the presence of aromatic DNA adducts in aluminum workers, and some preliminary data are presented in Table 2. The adduct levels decreased by about one half in the course of a 2–3 week summer vacation and tended to increase during a 5-day working period, indicating that they are job related. More data are being obtained, and their statistical evaluation is in progress. At present we cannot explain the differences in ELISA results between the coke oven and aluminum plant studies. A value of 27% positives in the coke-oven control group, which was recruited from a steel-rolling factory of the same plant, is in agreement with the values found by other investigators (Table 1), but is in sharp contrast to the result of the aluminum production plant where no PAH–DNA adducts in WBCs were detectable with the ELISA. It is possible that there was some exposure to PAH in the steel-rolling factory, but PAH exposure was not measured for this group (*11*).

Schoket et al. (*18*) analyzed peripheral blood lymphocytes (PBLs) from workers at two aluminum production plants in Hungary. The mean DNA adduct level obtained from workers of only one plant was significantly higher than that of the controls. The authors attributed this difference to differences in the technological design and different levels of exposure at the two plants. Vähäkangas et al. (*5*) examined the DNA samples from PBLs of 30 aluminum plant workers by SFS and found only one sample that contained a peak similar to BPDE–DNA. This result illustrates that BaP–DNA adduct levels in human WBCs are not sufficiently high to be detected by SFS.

Comparison of Data

Although we have used similar assays, and our data agree fairly well with those reported by other investigators, comparison of

results from different laboratories should be done with caution. Unfortunately, interlaboratory standardization of the methodology has not yet been realized, despite the fact that both immunoassays and the ^{32}P-postlabeling analysis have now been used for a period of 10 years. For example, the quantification of DNA adducts by ELISA depends on several factors, including modification level of standard DNA, characteristics of the particular antibody used, the type of assay, and the end point used. Underestimation of adducts in DNA from biological samples has been observed by us and other investigators using anti BPDE–DNA antisera and serially dilutions of highly modified BPDE–DNA as standards (*19,20*). Appropriate DNA preparations of low modification level, obtained from experimental animals treated with a specific PAH, are required as reference material when human DNA samples are to be analyzed.

Similar uncertainties are encountered in the quantification of adducts by the ^{32}P-postlabeling analysis. The postlabeling procedure is based on the relative resistance of the PAH-modified nucleotides to 3′-dephosphorylation by nuclease P1. However, various carcinogen–DNA adducts differ in their susceptibility to nuclease P1 (*21*). The micrococcal nuclease/spleen phosphodiesterase digestion used in the original ^{32}P-postlabeling procedure developed by Randerath failed to liberate deoxyadenosine adducts quantitatively (*22*). Thus, either degradation method can potentially lead to an underestimation of the extent to which specific DNA bases have been modified. Indeed, recent data from Shields et al. (*23*) suggest that previous ^{32}P-postlabeling studies for PAH–DNA adducts may have underestimated adduct levels. It is clear that in the future more emphasis should be placed on standardization of ^{32}P-postlabeling using internal standards, unmodified nucleotides as well as authentic PAH adducts, and combination with micropreparative techniques (HPLC) (*8,9,23*).

Relevance of White Blood Cells As Nontarget Cells

A correlation was found between PAH adduct levels in human lung and exposure to cigarette smoke (*24,25*), indicating that PAH–DNA adducts can be used as an indicator for internal exposure and risk assessment in target tissue. In human studies, however, target tissue samples can rarely be obtained. Blood samples are easily available, and the important question arises whether DNA adduct levels in WBCs reflect adduct levels in target tissues. If the persistence of the adducts in the target tissue and its surrogate differs too much, the existence of a correlation is unlikely. The use of WBCs as a nontarget tissue source of DNA was questioned by Phillips et al. (*26*). We did not find a correlation between PAH–DNA adduct levels in human lung and WBCs (*27*). Nontarget cells should be easily available and should have comparable properties with regard to carcinogen metabolism, DNA binding and repair, and turnover as the target cells. A useful nontarget source of DNA might be bronchoalveloar macrophages, obtained by bronchial lavage. Izzotti et al. (*28*) report that 85% of pulmonary alveolar macrophage samples from a group of current smokers showed typical BPDE–DNA fluorescence peaks when examined by SFS, whereas no adducts were detected in samples from nonsmokers or ex-smokers. Detailed knowledge on the above parameters for both target cell type and its surrogate might improve the utility of the latter. In this respect, more detailed studies on PAH–DNA adduct formation and repair in the various subpopulations of WBCs might be very helpful. Separation of WBCs followed by a 18-hr *in vitro* incubation with 10 μM BaP showed that the adduct level in long-lived monocytes and lymphocytes was at least 10 times higher than in short-lived granulocytes (*29*).

Interindividual and Intraindividual Variation

In all populations studied, substantial interindividual variation in PAH–DNA adduct levels has been observed. Although differences in exposure levels play a role, as outlined above, interindividual differences in PAH metabolism are also involved. The higher mean PAH–DNA adduct values (37 $\pm$ 95 adducts/10^8 nucleotides) we found in WBCs from lung cancer patients compared with those reported by Phillips et al. (*26*) for healthy smokers (5.53 $\pm$ 2.13 adducts/10^8 nucleotides) suggest a predictive value of PAH–DNA detection in WBCs. A disadvantage of the use of PAH–DNA adducts in WBCs is the substantial intraindividual variability. The majority of WBC (70–80%) are short-lived (hours to days), and the various cell types differ also in metabolizing capacity. Consequently, their contribution to DNA adduct formation will be different and variable. Recently, Thompson et al. (*30*) showed that human lymphocytes differed significantly in their ability to metabolize and bind PAH in complex mixtures, such as diesel organics, coke oven emissions, and cigarette smoke condensate. Separation of WBCs by physical methods or the use of immunocytochemistry may help to overcome the problem of intraindividual variation.

Sensitivity, Reproducibility, and Applicability

The high level of sensitivity of the ^{32}P-postlabeling assay with a lower detection limit of 1 adduct in 10^9 nucleotides is essential to measure adduct levels occurring in human DNA samples. The detection limit of the immunocytochemical detection we use is 7 BPDE–DNA adducts in 10^8 nucleotides. This method, with the aid of new quantification techniques (laser-scan microscopy), has promise for the measurement of adducts in human cells or tissues. Izzotti et al. (*27*) found PAH–DNA adduct levels of 1 adduct in 10^7 nucleotides in alveolar macrophages of smokers, levels that seem sufficient to be detected in individual cells by immunocytochemical methods. In summary, adduct-specific antibodies are quite useful in various specific applications, i.e., immunocytochemistry and enzyme immunoassays. As pointed out already, further standardization and validation of techniques is urgently needed to avoid systemic errors in measurements before these methods can be applied to large-scale epidemiological studies.

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