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Research Article

Bulky DNA Adducts in White Blood Cells: A Pooled Analysis of 3,600 Subjects

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Abstract

Background: Bulky DNA adducts are markers of exposure to genotoxic aromatic compounds, which reflect the ability of an individual to metabolically activate carcinogens and to repair DNA damage. Polycyclic aromatic hydrocarbons (PAHs) represent a major class of carcinogens that are capable of forming such adducts. Factors that have been reported to be related to DNA adduct levels include smoking, diet, body mass index (BMI), genetic polymorphisms, the season of collection of biologic material, and air pollutants.

Methods: We pooled 11 studies (3,600 subjects) in which bulky DNA adducts were measured in human white blood cells with similar ^{32}P-postlabeling techniques and for which a similar set of variables was available, including individual data on age, gender, ethnicity, batch, smoking habits, BMI, and season of blood collection, and a limited set of gene variants.

Results: Lowest DNA adduct levels ($P = 0.006$) were observed in the spring (median = 0.50 adducts per 10^8 nucleotides), followed by summer (0.64), autumn (0.70), and winter (0.85). The same pattern emerged in multivariate analysis but only among never smokers ($P = 0.02$). Adduct levels were significantly lower ($P = 0.001$) in northern Europe (the Netherlands and Denmark; mean = 0.60, median = 0.40) than in southern Europe (Italy, Spain, France, and Greece; mean = 0.79, median = 0.60).

Conclusions: In this large pooled analysis, we have found only weak associations between bulky DNA adducts and exposure variables. Seasonality (with higher adducts levels in winter) and air pollution may partly explain some of the interarea differences (north *vs*. south Europe), but most inter-area and inter-individual variations in adduct levels still remain unexplained.

Impact: Our study describes the largest pooled analysis of bulky DNA adducts so far, showing that interindividual variation is still largely unexplained, though seasonality seems to play a role. *Cancer Epidemiol Biomarkers Prev; 19(12); 3174–81. ©2010 AACR.*

Introduction

Bulky DNA adducts are markers both of exposure to genotoxic aromatic compounds and of the ability of the individual to metabolically activate carcinogens and to repair DNA damage (1). Experimental studies in animal models have highlighted the central role of DNA adduct formation in tumorigenesis (2), and key human studies have shown that carcinogenic polycyclic aromatic hydrocarbons (PAHs) represent a major class of carcinogens present in the environment and that are capable of forming DNA adducts at the same DNA bases in which p53 mutations are found in lung cells of smokers (3). When unrepaired, DNA adducts can cause mutations, including mutational hotspots in the *p53* tumor suppressor gene and other genes, which may ultimately induce cancer formation (3).

Human studies have shown a dose–response relationship between occupational exposure to PAHs and the levels of DNA adducts in lymphocytes of workers (4), but at high levels of exposure, saturation seems to occur. Although cigarette smoke also contains PAHs and other DNA adduct-forming compounds, studies on the association between tobacco smoking and DNA adducts in white blood cells (WBC) have yielded inconsistent results

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Table 1. Description of the studies

Name (references)	Population	*n* (%) of men[a]	Cells	Smoking habits
EPIC Spain (16, 17)	Spain	296 (50.34)	WBC	NS 174 EX 48 SM 74
Denmark study (20)	Denmark	255 (53.7)	WBC (BE)	NS 9 EX 56 SM 185 5 missing
Turin Bladder Case Control study (8, 21) Unpublished results	Italy	104 (100)	WBC	NS 29 EX 59 SM 24
EPIC Italy (9, 22)	Italy	634 (76.3)	WBC	NS 255 EX 204 SM 171 4 missing
GENAIR (12)	West Europe	1,086 (51.75)	WBC	NS 593 EX 492 1 missing
U.S. study (23, 24)	USA	173 (100)	WBC	NS 32 EX 72 SM 67 2 missing
Greece study (18)	Greece	194 (30)	Lymph	NS 194
The Netherlands study (19)	The Netherlands	41 (34.14)	Lymph	NS 5 SM 35 1 missing
Czech Republic study (11, 25, 26) Unpublished results	Czech Republic	360 (100)	Lymph	NS 330 SM 60
East Europe study (27, 28)	East Europe	354 (100)	Lymph	NS 212 SM 137 5 missing
Spain study (29)	Spain	76 (93)	Lymph	NS 31 EX 45
Total		3,573		

Abbreviations: lymph, lymphocytes; NS, never smokers; EX, ex-smokers; SM, current smokers; BE, butanol enrichment.
[a]From published work.

(5). In contrast, studies conducted on human lung tissue did show an association with tobacco smoke (4–7). Some studies have reported a negative correlation between DNA adduct levels and the consumption of fruit and vegetables and the intake of flavonoids (8–11), and the dose–response relationship with smoking may be affected by various dietary factors, especially in subjects with certain genetic polymorphisms in metabolic enzymes (5). Other factors that were reported to influence DNA adduct formation included body mass index (BMI), genetic polymorphisms in genes involved in the metabolism of carcinogens, the season in which the WBC/lymphocytes were sampled, and environmental pollutants such as O_3 and particulate matter (PM; refs. 4, 6, 12–14). A study undertaken in New York City after the events of September 11, 2001, found a direct relationship between the amount of DNA adducts in umbilical cord blood of newborn children and proximity to the World Trade Center (15), which suggests that air pollution may be a significant contributor to the formation of DNA adducts in blood.

Seasonality in DNA adduct levels has been observed and may be linked to the variability in air quality and human behavior, determining exposure between, for instance, summer and winter. The same variability with season could also be attributable to dietary habits. It is still insufficiently clear which factors contribute to the large interindividual variation in DNA adduct levels that is observed even when people are apparently exposed to similar doses of genotoxins.

Therefore, we have conducted a large pooled analysis in healthy individuals (~3,600 subjects) recruited in the context of case–control, cross-sectional, or cohort studies, with the purpose of validating or refuting previous findings in a sufficiently powered data set (8, 9, 11, 12, 16–29).

Methods

We have identified 11 study cohorts, investigated in 18 publications, listed in Table 1, in which bulky DNA adducts were measured by ^{32}P-postlabeling (41), and a similar set of variables was available, including individual data on age, gender, ethnicity, batch, smoking habits, BMI, and season of blood collection, and a limited set of gene variants. We contacted the principal investigators of these studies and had access to the original data sets. The study characteristics are briefly described in Table 1.

In most of the studies, measurement of bulky adducts by ^{32}P-postlabeling was achieved using the nuclease P1 digestion method of enrichment, although butanol extraction was used in the study of Bak and coworkers (20). In each investigation, subjects were enrolled after signing informed consent. Data sets were transferred to the ISI Foundation for analysis after being anonymized.

There were some differences in the mean levels of adducts among the studies, with the U.S. study showing the highest values (23, 24). This is most likely due to interlaboratory differences rather than to actual, exposure-related differences in DNA adduct levels, which have been expressed in the text as RAL (relative adduct labeling) $\times$ 10^8 bases, if not specified otherwise. We addressed this problem in 3 ways: 1) in the main analysis, data were normalized after pooling, assuming different measurement units in the different laboratories, according to the following formula:

$$\mathrm{RAL}_{st} = \frac{(\mathrm{RAL} - \mathrm{Mean}_{ic})}{\mathrm{SD}_{ic}}$$

where RAL is the relative adduct labeling; and Mean_{ic} and SD_{ic} mean and standard deviation of the group of subjects in the *i*th study. The rationale for using normalized values

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Table 2. Studies included in the analyses

Study	No. of subjects[a]	RAL	
		Mean	SD
EPIC Spain (WBC)	296	0.83	0.66
Denmark study (WBC)	255	0.23	0.15
Turin Bladder Case Control study (WBC)	104	0.43	0.50
EPIC Italy (WBC)	634	0.78	1.00
GENAIR (WBC)	1,086	0.70	0.55
U.S. study (WBC)	173	6.85	12.56
Greece study (L)	194	1.22	0.89
The Netherlands study (L)	41	1.53	0.56
Czech Republic study (L)	420	1.48	0.85
East Europe study (L)	354	1.06	0.40
Spain study (L)	76	0.23	0.58
Total	3,633	1.13	3.12

Abbreviations: WBC, white blood cells (buffy coat); L, lymphocytes.
[a]Mean values of RAL expressed as adducts per 10^8 nucleotides.

and quartiles to standardize genetic pooled analysis has been put forward previously by several authors and this approach has become common practice (4, 30–32). Because after standardization, the skewness of the distribution of RAL_{st} was still high (2.9), we compared standardized values of adduct levels using the nonparametric Kruskal–Wallis test; 2) we repeated all the statistical analyses excluding the study from the United States in which DNA adduct data were on average 8-fold higher than in the other studies (see Table 2); 3) for those studies in which DNA adduct analyses were conducted in different laboratories but using samples from the same populations (EPIC Spain and GENAIR in Spanish populations and Turin Bladder Case Control study, EPIC Italy, and GENAIR in Italian populations), we have applied analysis of variance (ANOVA) to compare the area effect with the laboratory effect.

In addition to descriptive statistics and ANOVA, we stratified univariate analyses and multivariate regression models by smoking habits, excluding those studies in which blood samples were not collected in all seasons (Greece, Czech Republic, and East Europe studies). In the multivariate model, we included sex, age, and seasonality. To control for heterogeneity among studies, we also considered multivariate regression models including the variable "study" as having a random effect. Finally, we conducted a logistic regression analysis in which the response variable was 0 or 1 if the RAL value was below or above the median value, respectively.

All statistical analyses were conducted using SAS software (v.9.1.3).

Results

Table 2 shows the mean (SD) adduct levels for the studies that were included in the analysis. There are relatively small variations among the studies except for the U.S. cohort that has adduct levels about a factor 8 higher than others. For this reason in the subsequent analyses, we use normalized levels. No statistically significant difference in DNA adduct levels with gender and BMI was observed (Table 3). Age showed a borderline significant association ($P = 0.09$), although no clear trend was observed. Seasonality (i.e., the season in which blood was drawn) and smoking (with higher levels in never smokers) were significantly associated with DNA adducts, $P = 0.006$ and 0.0003, respectively. Among the genetic variants that were analyzed in these studies, no statistically significant difference in DNA adduct levels with the variant genotypes was found (Table 4).

To verify whether the finding on smoking is true and not an artificial effect due to the statistical correction, we stratified the analysis between studies in which DNA adducts were measured in WBC and those in which they were measured in lymphocytes and we obtained the same trend as in the global analysis.

In the stratified multivariate analysis (Table 5), we observed an effect of seasonality in nonsmokers, with the lowest levels in the spring ($P = 0.02$), and an effect of sex, with women having higher levels, among current smokers ($P = 0.01$). The corresponding odds ratios (above *vs*. below the adduct median) were 0.74 (95% CI = 0.52–1.04) for spring *vs*. winter and 1.40 (95% CI = 0.97–2.00) for women *vs*. men. The R^2 (a measure of variance explained by the model) was very small for all models presented, always less than 0.02. Multivariate analysis for smoking showed a significant negative β value (−0.086, $P < 0.001$). Multivariate regression analysis including the variable "study" as having a random effect showed essentially similar results. ANOVA was conducted separately for the recruitment centers for which subpopulations were analyzed in different laboratories or in the same

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Table 3. Median RAL values according to selected individual characteristics

	No. of subjects	Median RAL (SD)	*P*
Sex (all)			
Male	2,352	0.83 (3.83)	0.65
Female	1,281	0.60 (0.79)	
Age (all)			
1 quartile	905	1.01 (0.83)	0.09
2 quartile	945	0.70 (1.29)	
3 quartile	872	0.60 (3.29)	
4 quartile	909	0.50 (5.12)	
Season (all)			
Spring	696	0.50 (2.70)	0.006
Summer	599	0.64 (3.82)	
Autumn	764	0.70 (5.06)	
Winter	1,232	0.85 (1.12)	
BMI (EPIC Spain, Greece, GENAIR, EPIC Italy)			
1 quartile	532	0.69 (0.93)	0.91
2 quartile	533	0.60 (0.75)	
3 quartile	537	0.60 (0.70)	
4 quartile	535	0.60 (0.65)	
Smoking status (all)			
Never	1,771	0.88 (1.40)	0.0003
Ex	1,043	0.54 (5.28)	
Current	781	0.67 (1.58)	

NOTE: Univariate analysis. *P* value from the Kruskal–Wallis test, based on RAL standardized values.

Table 4. Median RAL values according to genetic data

	No. of subjects	Median RAL (SD)	*P*
CYP1A1M1 (EPIC Spain, GENAIR, U.S. study)			
Wt	1,216	0.70 (4.76)	0.68
Het	256	0.80 (4.74)	
Mut	15	0.60 (1.04)	
GSTM1 (EPIC Spain, the Netherlands study, GENAIR, U.S. study, Czech Republic study, East Europe study)			
Null	1,157	0.90 (2.58)	0.39
Present	1,071	0.94 (4.93)	
GSTT1 (EPIC Spain, Greece, Turin Bladder Case Control study, GENAIR, Czech Republic study, East Europe study)			
Null	775	0.91 (0.66)	0.21
Present	1,527	0.80 (0.75)	
MPO (Turin Bladder Case Control study, GENAIR)			
Wt	677	0.60 (0.52)	0.86
Het	406	0.50 (0.56)	
Mut	49	0.60 (0.79)	
NQO1 (Greece, GENAIR)			
Wt	769	0.67 (0.66)	0.87
Het	390	0.64 (0.60)	
Mut	47	0.60 (0.63)	

NOTE: Univariate analysis. *P* value from Kruskal–Wallis test, based on RAL standardized values.
Abbreviations: Wt, wildtype; Het, heterozygous; Mut, homozygous variant; CYP1A1M1, cytochrome P450, family 1, subfamily A, polypeptide 1, T6235C transition; GSTT1, glutathione S-transferase theta 1; GSTM1, glutathione S-transferase mu 1; NQO1, NAD(P)H dehydrogenase, quinone; MPO, myeloperoxidase.

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Table 5. Univariate and multivariate models

Independent variable	Nonsmokers				Ex-smokers				Current smokers			
	Parameter estimate	SE	*P*	Model R^2	Parameter estimate	SE	*P*	Model R^2	Parameter estimate	SE	*P*	Model R^2
Univariate analysis												
DNA adducts												
Sex (ref: male)	0.064	0.066	0.33	0.001	0.011	0.076	0.88	0.000	0.183	0.077	0.02	0.011
Age (continuous)	−0.001	0.004	0.72	0.000	−0.003	0.003	0.33	0.001	0.000	0.005	0.99	0.000
Season				0.008				0.003				0.000
Spring	−0.218	0.092	0.02		−0.098	0.099	0.32		0.031	0.102	0.76	
Summer	−0.081	0.095	0.39		0.044	0.098	0.65		0.094	0.113	0.40	
Autumn	−0.001	0.091	0.99		0.001	0.099	0.99		0.117	0.103	0.25	
CYP1A1M1	−0.021	0.089	0.81	0.000	0.133	0.107	0.21	0.003	0.021	0.117	0.86	0.000
GSTM1	0.040	0.078	0.61	0.000	0.095	0.082	0.25	0.002	0.079	0.101	0.44	0.005
GSTT1	0.013	0.097	0.89	0.000	−0.062	0.098	0.530	0.001	0.118	0.210	0.570	0.004
MPO	0.031	0.081	0.70	0.000	−0.057	0.069	0.41	0.002	−0.075	0.197	0.71	0.010
NQO1	0.032	0.009	0.71	0.000	−0.068	0.073	0.36	0.002	–	–	–	–
Multivariate model												
DNA adducts				0.009				0.003				0.015
Age (continuous)	−0.002	0.004	0.74		−0.003	0.004	0.39		0.001	0.005	0.86	
Sex	0.062	0.066	0.35		−0.016	0.078	0.84		0.192	0.078	0.01	
Season												
Spring	−0.220	0.092	0.02		−0.096	0.099	0.33		0.011	0.102	0.91	
Summer	−0.085	0.095	0.37		0.040	0.099	0.69		0.097	0.112	0.39	
Autumn	−0.006	0.092	0.95		0.002	0.099	0.99		0.122	0.103	0.24	

NOTE: DNA adducts: dependent variable (standardized values).

Abbreviations: CYP1A1M1, cytochrome P450, family 1, subfamily A, polypeptide 1, T6235C transition; GSTT1, glutathione S-transferase theta 1; GSTM1, glutathione S-transferase mu 1; NQO1, NAD(P)H dehydrogenase, quinone; MPO, myeloperoxidase.

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laboratory at different times (simulating a batch effect; EPIC Spain, Turin Bladder Case Control Study, EPIC Italy, and GENAIR). The effect of center was greater than the effect of batch or laboratory ($F = 9.26$, $P < 0.0001$ for center; $F = 6.65$, $P = 0.0002$ for laboratory).

We also analyzed the nonstandardized RAL values, adjusting for laboratory effect and cell type, across Europe. Adduct levels were 0.60 (median = 0.40, SD = 0.54) in northern Europe (the Netherlands and Denmark) and 0.79 (median = 0.60, SD = 0.84) in southern Europe (Italy, Spain, France, and Greece), with a P value for the difference of 0.001.

Discussion

PAHs are an important class of environmental carcinogens, capable of inducing DNA adducts after metabolic activation (33). These may occur in fried and charcoal-grilled meat or in the food chain as a result of environmental pollution (34–36). As a result, human exposure to PAHs is widespread and may occur via inhalation, ingestion, or via dermal contact. The latter seems less relevant for the general population but may be of relevance in certain groups such as in occupational settings or after treatment with coal tar ointments. These exposures are thought to contribute to cancer incidence in the general population, as the most important targets for PAH carcinogenicity include lung and possibly bladder (1). Some evidence has also been reported for an association between dietary PAHs and colon cancer or adenomas (37, 38). Increased levels of bulky DNA adducts have been detected in the colon mucosa of colon cancer patients and in early stages of colon carcinogenesis (39, 40). More thorough understanding of factors that determine DNA adduct levels may thus contribute to improved preventive measures.

The ^{32}P-postlabeling assay is a complex procedure involving several steps (41). Although guideline protocols have been devised and tested in interlaboratory trials (42), there is no consensus on conditions for analysis or methods for quantitation. For the latter, differences between studies may reside in how DNA adduct levels are calculated from the levels of radioactivity detected on thin-layer chromatography (TLC) plates; different approaches include separate assessment of the incorporation of radioactivity into normal nucleotides or determination of the specific activity of the [γ-^{32}P]ATP used. It is also not clear which areas of the TLC plates were included in the quantitation; this can be of some importance in cases, such as here, in which DNA adduct patterns may be weak and diffuse. For the purposes of pooled analysis, however, interlaboratory differences can be accommodated by normalizing results, as was done in the present study.

The present study is the largest pooled analysis available on bulky DNA adducts (~3,600 subjects) and shows only weak associations. The analysis restricted to studies having data for every season confirms an association with the season at the time of blood collection, as suggested in previous smaller studies. In nonsmokers, we found significantly lower DNA adduct levels in spring ($P = 0.02$) than in winter, with a seasonal gradient similar to the one shown for median levels in Table 3. This may have 2 alternative explanations: the first is a protective effect of seasonal dietary intakes such as fresh fruit and vegetables, although this is less likely to peak in the spring when the lowest RAL were observed. Such a protection has been suggested in previous investigations (8–11) but could not be tested directly in the current analysis because the data sets are too heterogeneous in the way dietary data were collected. The second potential explanation is a higher level of bulky adducts in some seasons due to higher levels of exposure to pollution, particularly to particulate-bound PAHs. This can be due to seasonal differences in emissions, weather conditions, and/or outdoor human activity. This hypothesis seems to be supported by some of the previous investigations (33) and is confirmed by a comparison among the areas for which we had adduct measures from different laboratories. In fact, after adjusting for the laboratory effect and cell type, mean adduct levels were 0.60 (median = 0.40, SD = 0.54) in northern Europe (the Netherlands and Denmark) and 0.79 (median = 0.60, SD = 0.84) in southern Europe (Italy, Spain, France, and Greece), a trend that corresponds to the different levels of PM2.5, PM10, and NO_2 that have been observed across Europe. According to a recent comprehensive report, PM2.5 concentrations, for example, are clearly greater in cities from southern Europe (with peaks of >40 mg/m^3 in Turin, Italy) than in cities form northern Europe (43).

The observation of lower adducts in smokers compared with nonsmokers, is counterintuitive. A first observation can be that current smokers are less represented in our sample. Moreover, nucleotide excision repair capacity is one of the factors that could contribute to individual variation in tobacco-related biomarkers. Previous studies have shown that smokers (particularly current smokers) tended to have more proficient DNA repair capacity (DRC) than nonsmokers, suggesting that smokers may have an adaptive response to DNA damage induced in blood cells by chronic tobacco carcinogen exposure. In particular, higher DRC was shown in smokers in *in vitro*–induced Benzo[a]pyrene diol epoxide (BPDE)-adduct repair (44); in oxidative damage repair (45, 46); in 4-aminobiphenyl adduct repair, also related to smoking habits (47); and in the γ-radiation repair model (48). The hypothesis that the induction of DNA damage by smoking can stimulate cellular repair activity could explain the significantly higher DNA adduct levels in nonsmokers than in smokers ($P = 0.0003$) in our pooled analysis.

Recently, it has been shown that phase II enzymes can be induced by PAHs found in cigarette smoke (49). These enzymes are involved in the process of detoxification of numerous carcinogens such as PAHs and aryl- and heterocyclic amines (50), and their induction by tobacco

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smoke could be an alternative explanation for the smoking effect in our study, in which preferential induction of phase II enzymes can lead to more rapid clearance of PAHs prior to adduct formation. Moreover, interindividual differences exist in the levels of expression and catalytic activities of a variety of xenobiotic-metabolizing enzymes in humans and these phenomena are thought to be critical in understanding the basis of different susceptibilities of individuals to PAH action (51).

Conclusions

In this large pooled analysis, we have reported only weak associations between bulky DNA adducts and exposure variables, namely, seasonality. Most comparisons were negative and also the R^2 of all regression models was extremely small (>0.02), suggesting that the part of variance explained by these models is very modest. Air pollution may partly explain some of the inter-area differences (between north and south Europe), but most inter-area and inter-individual variations in adduct levels still remain unexplained.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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