Relationship between Ambient Air Pollution and DNA Damage in Polish Mothers and Newborns

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Industrialized regions in Poland are characterized by high ambient pollution, including polycyclic aromatic hydrocarbons (PAHs) from coal burning for industry and home heating. In experimental bioassays, certain PAHs are transplacental carcinogens and developmental toxicants. Biologic markers can facilitate evaluation of effects of environmental PAHs on the developing infant. We measured the amount of PAHs bound to DNA (PAH–DNA adducts) in maternal and umbilical white blood cells. The cohort consisted of 70 mothers and newborns from Krakow, Poland, an industrialized city with elevated air pollution. Modulation of adduct levels by genotypes previously linked to risk of lung cancer, specifically glutathione *S*-transferase M1 (GSTM1) and cytochrome P4501A1 (*CYP1A1*) *Msp*I restriction fragment length polymorphism (RFLP), was also investigated. There was a dose-related increase in maternal and newborn adduct levels with ambient pollution at the women's place of residence among subjects who were not employed away from home ($p \leq 0.05$). Maternal smoking (active and passive) significantly increased maternal ($p \leq 0.01$) but not newborn adduct levels. Neither *CYP1A1 Msp*I nor GSTM1 polymorphisms was associated with maternal adducts. However, adducts were significantly higher in newborns heterozygous or homozygous for the *CYP1A1 Msp*I RFLP compared to newborns without the RFLP ($p = 0.04$). Results indicate that PAH-induced DNA damage in mothers and newborns is increased by ambient air pollution. In the fetus, this damage appears to be enhanced by the *CYP1A1 Msp*I polymorphism. — *Environ Health Perspect* 106(Suppl 3):821–826 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-3/821-826whyatt/abstract.html*

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Introduction

Ambient air in certain industrialized regions of Poland is heavily contaminated with polycyclic aromatic hydrocarbons (PAHs) (*1*). Krakow is an industrialized city with high air pollution attributed to multiple sources, especially coal burning for industrial purposes and residential heating (*2*). Pollution levels are highest in the center of Krakow and decrease toward the periphery. A previous study found lung cancer risk to be significantly associated with residence in the highest air pollution areas of Krakow, controlling for age, smoking, and occupational exposures (*2*). For the current cohort, we estimate that in the year preceding the birth of the newborns (1991), the women living in Krakow were exposed to annual average ambient concentrations of respirable particulates ranging from 37 $\mu g/m^3$ for the least exposed group to 78 $\mu g/m^3$ for the most exposed. The corresponding concentrations of benzo[*a*]pyrene, an indicator PAH, were estimated to be 7 ng/m^3 to 15 ng/m^3, representing approximately 0.02% of particulate matter (*3*).

PAHs readily cross the placenta (*4,5*). Experimental bioassays have shown a number of PAHs to be transplacental carcinogens and developmental toxicants (*6–11*). PAH–DNA adducts represent the net effect of exposure, absorption, activation, detoxification, and repair and thus are a better measure of the individual biologically effective dose of PAHs than estimates of external exposure (*12,13*). PAHs are rapidly distributed systemically (*14*), and comparable levels of PAH–DNA adducts across many tissues, including peripheral blood, have been seen in experimental and human studies (*15,16*). Depending on the degree of damage or repair capacity, DNA adducts can be repaired or lead to apoptosis or mutations. A correlation has been observed experimentally between carcinogenicity and adduct formation for a series of mutagens and carcinogens, including PAHs (*17–19*). A recent case–control study showed white blood cell (WBC) PAH–DNA adduct levels to be associated with increased risk of lung cancer (*16*). Among the current cohort, we reported an association between newborn WBC PAH–DNA adduct levels and adverse birth outcomes (*20*). Other studies have seen associations between WBC adduct levels and ambient PAH levels measured by areawide (*1*) and personal (*21*) monitoring.

Genetic differences in detoxification capabilities may modulate PAH-induced

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Abbreviations used: +, positive; –, negative; AHH, aryl hydrocarbon hydroxylase; ELISA, enzyme-linked immunosorbent assay; EROD, 7-ethoxyresorufin *O*-deethylase; ETS, environmental tobacco smoke; PAH, polycyclic aromatic hydrocarbon; PCR, polymerase chain reaction; PM_{10}, ambient particulates < 10 μm in aerodynamic diameter; RFLP, restriction fragment length polymorphism; WBC, white blood cell.

carcinogenesis (*12*). CYP1A1 is an inducible enzyme system that catalyzes the biotransformation of PAHs to phenolic products and epoxides (*22,23*). These can be further biotransformed by epoxide hydrolase and other enzymes to reactive metabolites capable of binding to DNA (*24*). A *Msp*I restriction fragment length polymorphism (RFLP) has been identified in the 3′ noncoding region of the *CYP1A1* gene (the *CYP1A1 Msp*I RFLP). It segregates in linkage disequilibrium with a polymorphism in exon 7 that results in an Ile → Val substitution in the catalytic region. Both *CYP1A1* polymorphisms have been associated with lung cancer risk in some, but not all, studies (*25–31*).

GSTM1 codes for an enzyme involved in the detoxification of PAHs via conjugation of activated metabolites with glutathione. An estimated 30 to 60% of populations are deleted at this locus (*32*). The GSTM1 null genotype has been associated with increased risk of lung cancer (*16,33–35*) albeit not consistently (*36–38*). Previous evaluations of the association between the *CYP1A1* and GSTM1 polymorphisms and carcinogen–DNA adduct levels have provided conflicting results (*39–43*).

The current study extends previous research in Poland (*1*) by investigating the association between ambient air pollution and PAH–DNA adduct formation in mothers and newborns. The study also evaluates whether the *CYP1A1 Msp*I RFLP modulates adduct levels in maternal and newborn WBCs and whether GSTM1 influences maternal adduct levels. GSTM1 is expressed rarely and only at low levels in fetal tissues (*44*) and was not hypothesized to affect DNA damage in the newborn.

Materials and Methods

Study Subjects and Data Collection

Field studies were conducted during the winter of 1992 under the direction of W. Jedrychowski (Jagiellonian University, Krakow, Poland). The cohort consisted of 70 mother–newborn pairs from Krakow. Samples of umbilical cord blood and placental tissue were collected immediately after birth and a sample of maternal blood was obtained within 2 days postpartum; biologic samples were processed and stored as described previously (*3*).

A detailed validated questionnaire administered to the mother within 2 days postpartum included information on smoking (active and passive), residential and employment histories, use of coal stoves for residential heating, dietary sources of PAHs, and residential or occupational exposures to PAHs and inducers of *CYP1A1* (*3*). All interviews were conducted by two trained interviewers from the College of Medicine, Jagiellonian University. Coded questionnaire data were sent to Columbia University (New York, NY). Assessment of smoking status was based on questionnaire data as described previously (*3*). Current smokers were defined as having smoked one or more cigarettes/day for 6 months or more during their lifetimes and were smoking up to delivery. Ex-smokers were defined as having smoked one or more cigarettes/day for 6 months or more during their lifetimes but having quit 1 month or more prior to delivery. Ex-smokers were further divided into those who had quit prior to and those who quit during pregnancy. Plasma cotinine (a marker of recent cigarette smoke exposure) was used to verify questionnaire data as described previously (*3*).

Daily ambient monitoring data were provided for Krakow for the period 1991 to 1992 by the Division of National Sanitary Inspection (Krakow) (15 monitoring stations) and by the U.S. Environmental Protection Agency (5 monitoring stations). Each Krakow woman's exposure to ambient particulates < 10 μm in aerodynamic diameter (PM_{10}) was estimated by taking the average of PM_{10} measurements (in micrograms per cubic meter) reported at the monitoring station closest to her residence for the year prior to her delivery date. Ambient particulate data were available for 69 of 70 subjects from Krakow.

PAH–DNA Adducts by Competitive Enzyme-Linked Immunosorbent Assay

DNA was extracted from maternal and umbilical cord WBCs and PAH–DNA adducts were measured by a competitive enzyme-linked immunosorbent assay (ELISA) with fluorescence end point detection essentially as described previously (*45*). The antiserum was generated in a rabbit immunized with benzo[*a*]pyrene diol-epoxide (BPDE)–DNA but it recognizes other structurally related PAH diol epoxide–DNA adducts, including those formed by benz[*a*]anthracene and chrysene (*46*). Thus positive reaction with the antiserum may indicate the presence of multiple PAH–DNA adducts in the sample; values are expressed as the amount of BPDE–DNA that would cause a similar inhibition in the assay. The quantity of DNA was adequate to measure PAH–DNA adduct levels in 57 maternal and 58 umbilical cord blood samples. These measurements included adduct levels in 45 mother–newborn pairs.

*CYP1A1 Msp*I RFLP

The polymerase chain reaction (PCR)-RFLP method was used to determine *CYP1A1 Msp*I genotype using DNA from umbilical cord samples as described previously (*3*). High-molecular-weight genomic DNA from placental villus (fetal) samples was also digested with *Msp*I and resultant fragments were electrophoretically separated and visualized by autoradiography following hybridization with radiolabeled cDNA probes as described (*3*). Determination of the *CYP1A1 Msp*I RFLP was completed on 61 umbilical cord DNA samples and 62 placenta samples. For those subjects in which the RFLP was determined by both methods, concordance was 100%.

GSTM1 Genotype

DNA samples were genotyped by a PCR method (*32*). Subjects were categorized as either positive (+) or negative (–). For all analyses, + and – control reactions were run in parallel.

Plasma Cotinine

Levels of cotinine were measured in maternal and umbilical cord plasma using gas chromatography as previously described (*3*).

Statistical Analyses

PAH–DNA adduct levels in maternal and newborn WBCs were log-transformed to stabilize the variance and obtain a more symmetrical distribution. For samples with nondetectable adduct levels, a value of half the detection limit was assigned prior to transformation. Means and standard errors are presented as untransformed values for ease of interpretation. Associations were examined by multiple linear regression ($\alpha = 0.05$). Regression models controlled for cigarette smoking status, number of servings per week of foods high in PAHs during pregnancy, use of coal stoves for residential heating, and home or occupational exposures to PAHs and other organics. Krakow subjects were divided into low, medium, and high pollution groups based on estimated average ambient PM_{10} levels at the woman's place of residence during the year prior to delivery. The difference in adduct levels across pollution groups was determined for all Krakow subjects and for the subset of Krakow subjects ($n = 25$) not

employed away from home during pregnancy. Although sample size was limited, estimates of exposure for the latter group are considered more reliable because unemployed women spend more of their time at their place of residence. Correlations between biomarkers were assessed by Spearman's rank order correlation test. Differences in adduct levels in paired maternal and newborn samples were assessed by the Wilcoxon signed ranks test.

Results

Maternal age and smoking status are reported in Table 1. Mean PAH–DNA adduct levels were similar in paired maternal (6.4 ± 1.4 per 10^8 nucleotides) and newborn (6.2 ± 1.3 per 10^8 nucleotides) WBCs ($n = 45$), but there was not a significant correlation between maternal and newborn adduct levels ($r = 0.19$, $p = 0.2$).

PAH–DNA adduct levels (unadjusted means) in maternal and newborn WBCs by ambient air pollution group, smoking categories, and genotype are presented in Table 2. Table 3 shows the results of multivariate analyses; Figure 1 presents adjusted geometric mean adduct levels by ambient pollution group for Krakow subjects not employed away from home. Estimated ambient concentrations of PM_{10} for Krakow subjects at their place of residence during the year prior to delivery averaged 54 μg/m^3 (range 31–97 μg/m^3). When all Krakow subjects were divided into low, medium, and high pollution groups based on the estimated PM_{10} levels, no difference in adduct levels was seen across pollution groups. When analyses were restricted to Krakow mothers not employed away from home, WBC adduct levels were significantly increased in women residing in the high as compared to the low pollution group ($p = 0.05$). Among the newborns of unemployed women, WBC adduct levels were significantly higher in newborns residing in both the middle ($p = 0.05$) and high ($p = 0.03$) pollution areas as compared to the low pollution area.

Maternal WBC PAH–DNA adduct levels were significantly higher in current smokers compared to both nonsmokers and ex-smokers (Table 3), including ex-smokers who quit during pregnancy ($p < 0.01$, $n = 11$) as well as those who quit before pregnancy ($p < 0.05$, $n = 8$). Among the current smokers, no association was seen between self-reported number of cigarettes the women smoked per day during pregnancy and maternal adduct levels (Table 3). Among nonsmokers, maternal WBC adducts were significantly higher in subjects reporting environmental tobacco smoke (ETS) exposure as compared to those reporting no ETS exposure (Table 3). No association was seen between maternal adduct levels and self-reported number of cigarettes per day of passive exposure during pregnancy. Further, no association was seen between maternal WBC adduct levels and maternal plasma cotinine levels.

Contrary to results with maternal WBC adduct levels, newborn WBC PAH–DNA adduct levels were not associated with either active or passive smoking status of the mother or the number of

Table 1. Age and cigarette smoking status of mothers ($n = 70$).

Mother's age, years	27.6 ± 5.3[a]
Current smokers	12[b]
Cigarettes/day during pregnancy, current smokers	8.6 (range 2–30)[a]
Ex-smokers	20[b]
Nonsmokers	38[b]
ETS exposure, nonsmokers	22[b]
Passive cigarettes/day during pregnancy, nonsmokers	9.6 (range 1–30)[a]

ETS, environmental tobacco smoke. [a]Mean ± standard deviation (range). [b]Number of subjects.

Table 2. White blood cell PAH–DNA adduct levels[a] for Krakow mothers and newborns by ambient pollution group, cigarette smoking status (active and passive), and genotype.

Exposure and genotype	Maternal	Newborn
Pollution group, Krakow total		
Low	5.9 ± 2.1 (20)	5.6 ± 2.1 (17)
Medium	7.6 ± 2.7 (19)	6.9 ± 2.1 (20)
High	7.1 ± 2.1 (17)	5.0 ± 1.2 (20)
Pollution group, Krakow unemployed[b]		
Low	2.6 ± 1.2 (5)	1.7 ± 0.5 (6)
Medium	5.2 ± 2.3 (8)	9.6 ± 3.5 (9)
High	11.1 ± 5.3 (6)	6.5 ± 2.0 (8)
Active smoking status		
Nonsmoker	6.9 ± 1.7 (26)	5.6 ± 1.2 (32)
Ex-smoker	3.0 ± 0.5 (19)	5.9 ± 2.1 (19)
Current smoker	12.4 ± 4.5 (12)	6.1 ± 3.7 (7)
Passive smoking status, nonsmokers		
ETS–	3.3 ± 1.0 (10)	4.9 ± 1.8 (15)
ETS+	9.1 ± 2.6 (16)	6.1 ± 1.7 (17)
*CYP1A1 Msp*I RFLP		
*Msp*I –/–	7.1 ± 1.7 (43)	4.8 ± 1.0 (43)
*Msp*I –/+, +/+	4.9 ± 1.2 (12)	8.7 ± 2.8 (14)

[a]Unadjusted mean ± standard error per 10^8 nucleotides (n). [b]Exposure estimates are more reliable for this group because they are based on data from monitoring stations nearest the residence.

Table 3. Multiple linear regression analyses.[a]

	Maternal WBC PAH–DNA levels		Infant WBC PAH–DNA levels	
Exposure and genotype	Beta	p-Value	Beta	p-Value
Ambient pollution				
Ambient pollution group Krakow, total[b]	–0.04	0.91	0.09	0.84
Ambient pollution group Krakow, unemployed[b]	1.77	0.05	1.73	0.03
Cigarette smoke				
Current versus nonsmoker	1.03	<0.01	–0.20	0.70
Current versus ex-smoker	1.42	<0.01	–0.38	0.50
Ex-smoker versus nonsmoker	–0.33	0.27	–0.04	0.91
Cigarettes/day during pregnancy, current smokers	0.09	0.36	–0.01	0.97
Plasma cotinine, ng/ml	<0.01	0.21	–0.01	0.14
ETS+ versus ETS–, nonsmokers only	1.09	0.01	0.23	0.60
Passive cigarettes/day during pregnancy, nonsmokers only	<0.01	0.81	0.02	0.55
Genotype				
*CYP1A1 Msp*I–/+, +/+ versus *CYP1A1 Msp*I–/–	–0.13	0.72	0.81	0.04
GSTM1–/– versus GSTM1+/+, +/–	–0.02	0.95	–	–

[a]Associations between PAH–DNA adduct levels, environmental exposures (ambient pollution and cigarette smoke), and genotype (*CYP1A1 Msp*I and GSTM1) were evaluated by multiple linear regression; models controlled for smoking status, dietary PAH, use of coal stoves for residential heating, and home or occupational exposures to PAH and other organics. [b]High versus low exposure to PM_{10}.

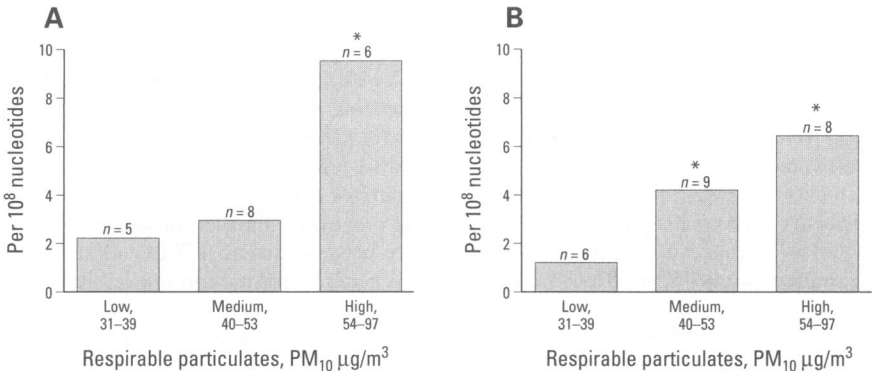

Figure 1. White blood cell PAH–DNA adducts[a] by level of air pollution in Krakow subjects not employed outside the home. [a]Geometric means adjusted by smoking status, dietary PAH, use of coal stoves for residential heating, and home or occupational exposures to PAH and other organics. (*A*) Mothers; (*B*) newborns. *$p \leq 0.05$ compared to low-pollution group.

cigarettes the mother smoked per day during pregnancy, nor was there an association between newborn adduct levels and either the number of cigarettes per day of passive exposure the mother reported during pregnancy or newborn plasma cotinine levels (nanogram per milliliter).

With respect to the modulation of WBC PAH–DNA adduct levels by genotype, no significant effect of either the mother's *CYP1A1 Msp*I RFLP or the GSTM1 genotype on maternal WBC adduct levels was apparent in maternal blood samples (Tables 2 and 3), nor was there an association between the mother's *CYP1A1 Msp*I RFLP or GSTM1 genotype and adduct levels in the newborn. However, in blood samples from the newborn, PAH–DNA adduct levels were significantly higher in WBCs of newborns who were heterozygous or homozygous for the *CYP1A1 Msp*I RFLP (*Msp*I–/+,+/+) as compared to newborns without the RFLP (*Msp*I–/–) ($p = 0.04$).

Discussion

Coal-burning furnaces used for industry and heating are a principal source of ambient pollution within Krakow. The heaviest pollution is found in the older central sections of the city. In the current study, WBC adduct levels were significantly increased in both mothers and newborns residing in the most polluted area, restricted to those women not employed away from home during pregnancy. Exposure estimates are most reliable for this group because they are based on data from monitoring stations nearest the women's residence. Although limited by the small sample size, this finding is consistent with a prior case–control study from Krakow in which a significant association was seen between lung cancer risk and residence in the high pollution area of Krakow (*2*). Few prior studies have evaluated associations between ambient air pollution and PAH–DNA binding in newborns. However, a significant association between air pollution and WBC–DNA adduct levels has been reported previously in adult populations (*1,21*).

Active and passive smoking status of the mother was also significantly associated with PAH–DNA adduct levels in maternal, but not newborn, WBCs. There are several explanations for the fact that the relationship between ambient air pollution and adduct levels was similar for maternal and newborn WBCs, whereas the relationship between cigarette smoke exposures and adduct levels differed for mother and newborns. Cigarette smoke may induce more Phase I metabolism in the maternal than in the fetal tissues, or induction of *CYP1A1* in placentas of smokers may modulate the biologically effective dose to the fetus (*47,48*). Consistent with our findings, previous studies of adult populations have seen significant associations between active smoking and WBC PAH–DNA adduct levels (*16,49,50*), although results have not been consistent (*51*). Several prior studies using the ^{32}P-postlabeling method, which measures a broad spectrum of adducts bound to DNA, have seen an association between maternal smoking and adduct levels in fetal samples (*52–54*). However, studies using methodologies specific to PAH–DNA adducts have not (*52,55,56*).

Prior evaluations of effects of ETS exposure on DNA damage are limited and most have not seen an association (*57–60*). However, we previously reported a significant increase in PAH–albumin adducts (a surrogate for PAH–DNA adducts) in children exposed to ETS (*61*). ETS exposure is high in Poland. A recent study of nonsmoking women from Poland found that the majority were exposed to ETS either at home or the workplace and that urinary cotinine was detected in 92% of nonsmoking women sampled (*62*). In the current study, 58% of the nonsmoking women reported ETS exposures.

To our knowledge, this is the first study to evaluate the association between the *CYP1A1 Msp*I RFLP and DNA damage in fetal samples. WBC adduct levels were significantly higher among newborns who were heterozygous or homozygous for the *CYP1A1 Msp*I RFLP compared to newborns without the RFLP. However, in maternal samples no association was seen between PAH–DNA adduct levels in maternal WBC and either GSTM1 or *CYP1A1* genotypes of the mother. Results of the relationship between adduct levels and the *CYP1A1 Msp*I RFLP and related exon 7 polymorphism (an Ile → Val substitution in the catalytic region) in other adult populations have been conflicting (*39,41*); however, an association between WBC PAH–DNA adduct levels and the exon 7 polymorphism was recently seen among smoking U.S. study subjects (*42*). Our results suggest that the *CYP1A1 Msp*I RFLP may be associated with greater DNA damage in fetal than maternal tissues. A possible mechanism for this difference in susceptibility is lack of fetal expression of GSTM1 (*44*), an enzyme system that facilitates conjugation and excretion of reactive PAH metabolites. Another is lower DNA repair efficiency in the fetus relative to the adult (*63–65*), rendering the fetus more sensitive to the effects of the genotype.

Some, but not all, prior studies have shown an association between lung cancer risk and both the *CYP1A1 Msp*I RFLP and exon 7 polymorphism (*25,26,31*). However, the mechanism(s) by which the polymorphisms could increase DNA adducts and lung cancer risk have not been elucidated (*66*). A possible mechanism is higher CYP1A1 inducibility or enhanced catalytic activity of the valine-type CYP1A1 enzyme (*67*). The exon 7 polymorphism has been associated with increased CYP1A1 activity (*68,69*), but not consistently (*70*). It is also possible that the polymorphisms are linked in certain populations to other mutations important in CYP1A1 inducibility (*66*).

Another novel feature of the current study is the measurement of WBC PAH-DNA adduct levels in mother–newborn pairs. Experimental bioassays indicate that transplacental exposures to PAHs are generally an order of magnitude lower than

maternal exposure (*4,5*). Therefore, the finding that levels in the newborns were similar to those in the mothers was not anticipated and suggests the possibility of increased susceptibility of the developing fetus to DNA damage. We separately analyzed PAH–DNA adduct levels from mothers and newborns in Limanowa, a rural area outside of Krakow where ambient pollution levels are lower but home use of coal for heating is significantly greater. Among the 67 mother–newborn pairs from Limanowa, mean adduct levels in the newborns significantly exceeded those in the mothers (9.0 ± 1.3 vs 5.6 ± 0.9 per 10^8 nucleotides) (*71*).

In conclusion, the evidence of significant genetic damage in newborns associated with environmental PAHs raises concern about carcinogenic risks from *in utero* exposure to this widespread contaminant.

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