Short Communication

In Utero DNA Damage from Environmental Pollution Is Associated with Somatic Gene Mutation in Newborns[1]

Frederica Perera,[2] Karl Hemminki, Wieslaw Jedrychowski, Robin Whyatt, Ulka Campbell, Yanzhi Hsu, Regina Santella, Richard Albertini, and James P. O'Neill

Columbia University School of Public Health, New York, New York 10032 [F. P., R. W., U. C., Y. H., R. S.]; College of Medicine, Jagiellonian University, Krakow 31-034, Poland [K. H., W. J.]; and University of Vermont Genetics Laboratory, Burlington, Vermont 05405 [J. P. O.]

Abstract

Transplacental exposure to carcinogenic air pollutants from the combustion of fossil fuels is a growing health concern, given evidence of the heightened susceptibility of the fetus. These mutagenic/carcinogenic pollutants include aromatic compounds such as polycyclic aromatic hydrocarbons that bind to DNA, forming chemical-DNA adducts. We have investigated the genotoxic effects of transplacental exposure in humans by analyzing aromatic-DNA adducts and the frequency of gene mutations at the *hypoxanthine-guanine phosphoribosyltransferase* (*HPRT*) locus in umbilical cord and maternal blood samples. Here we show, in a cross-sectional study of 67 mothers and 64 newborns from the Krakow Region of Poland, that aromatic-DNA adducts measured by ^{32}P-postlabeling are positively associated with *HPRT* mutant frequency in the newborns ($\beta = 0.56$, $P = 0.03$) after controlling for exposure to tobacco smoke, diet, and socioeconomic status. In contrast to the fetus, *HPRT* mutations and DNA adducts do not reflect similar exposure periods in the mother, and the maternal biomarkers were not correlated. Adducts were higher in the newborn than the mother, indicating differential susceptibility of the fetus to DNA damage; but *HPRT* mutation frequency was 4-fold lower, consistent with the long lifetime of the biomarker. These results provide the first demonstration of a molecular link between somatic mutation in the newborn and transplacental exposure to common air pollutants, a finding that is relevant to cancer risk assessment.

Introduction

There is mounting evidence of the differential susceptibility of the fetus to diverse environmental toxicants, including carcinogens (1–4). By incorporating biomarkers such as carcinogen-DNA adducts and gene mutation into human studies, molecular epidemiology has the potential to prevent disease both by elucidating disease mechanisms and by identifying exposed populations at increased risk of disease (5, 6). Both adducts and mutations have been associated with increased risk of cancer (7–10). We have reported previously that adults exposed to air pollution in Poland had increased levels of aromatic-DNA adducts and chromosomal aberrations in peripheral blood samples ($P \leq 0.01$; Ref. 11), and that aromatic adducts were significantly higher in Polish newborns than in mothers ($P = 0.002$), indicating greater susceptibility of the fetus to DNA damage (4).

To understand effects of transplacental environmental exposures on somatic cell mutations, we have used the assay for mutagenic events in the *HPRT*[3] reporter gene that is widely used for monitoring human populations for genotoxicity and potential carcinogenicity (12–14). The *HPRT* gene product is a phosphorybosylation enzyme in the purine salvage pathway that also phosphoribosylates purine analogues such as TG, resulting in cell lethality. This provides an effective system for mutant selection because only those cells with an inactivating *HPRT* mutation are able to proliferate in the presence of TG. Prior studies have established a significant correlation between *HPRT* MF and MF (15). Therefore, mutation frequency (per 10^6 cells) is considered to be a valid proxy for mutation frequency. We determined the relationship between *HPRT* MF and environmental exposures estimated both by questionnaire data (smoking, use of coal for indoor heating, and diet) and by biomarkers (DNA adducts and plasma cotinine as an internal dosimeter of tobacco smoke).

Materials and Methods

Study Area and Subjects. Subjects were 67 mothers and 64 newborns (48 pairs with *HPRT*) from Krakow and Limanowa, Poland, who participated in a larger study of transplacental exposure and birth outcomes and whose blood samples were adequate for *HPRT* analysis (16). Subjects were enrolled during the winter of 1992. The subset was representative of the parent population in terms of demographic and exposure characteristics. Krakow has elevated air pollution resulting from combustion of coal and from motor vehicle emissions, whereas the town of Limanowa has lower ambient pollution levels but increased burning of coal for home heating. The women from the two study areas did not differ significantly with respect to

Received 2/22/02; revised 5/15/02; accepted 6/14/02.
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[1] Supported in part by grants from National Institute of Environmental Health Sciences, United States Environmental Protection Agency, Department of Energy, Gladys and Roland Harriman Foundation, March of Dimes Birth Defects Foundation, W. Alton Jones Foundation, and New York Community Trust.
[2] To whom requests for reprints should be addressed, at Columbia University, School of Public Health, 60 Haven Avenue, B-109, New York, NY 10032. E-mail: Fpp1@columbia.edu.

[3] The abbreviations used are: HPRT, hypoxanthine-guanine phosphoribosyltransferase; TG, 6-thioguanine; MF, mutation frequency; PAH, polycyclic aromatic hydrocarbon; SES, socioeconomic status.

Table 1 Results for four biomarkers among the 48 paired Polish mothers and newborns with *HPRT*[a]

Exposure/Biomarker	Mothers		Newborns	
	n (%)	Mean (SE)	*n* (%)	Mean (SE)
Plasma cotinine (ng/ml)	48	12.1 (5.0)	48	17.9 (5.9)
High[b]	19 (39.6)	30.3 (50.2)	22 (45.8)	38.8 (53.4)
Low	29 (60.4)	0.25 (0)	26 (54.2)	0.25 (0)
PAH-DNA adducts (per 10^8 nucleotides)	40	6.1 (1.4)	44	10.0 (1.8)
High[b]	22 (55.0)	10.0 (10.3)	23 (52.3)	18.0 (12.1)
Low	18 (45.0)	1.3 (0.8)	21 (47.7)	1.3 (0.8)
Aromatic-DNA adducts (per 10^8 nucleotides)	44	15.0 (2.1)	46	19.3 (1.9)
High[b]	22 (50.0)	23.4 (15.3)	23 (50.0)	28.2 (11.7)
Low	22 (50.0)	6.6 (3.7)	23 (50.0)	10.4 (5.6)
HPRT MF (per 10^6 cells)	48	18.8 (2.7)	48	4.4 (1.2)

[a] By paired *t* test, cotinine and adducts were higher in newborns than mothers: cotinine ($P = 0.009$); PAH-DNA adducts ($P = 0.06$); and ^{32}P aromatic-DNA adducts ($P = 0.08$). *HPRT* MF was higher in mothers ($P < 0.0001$).
[b] High/low based on median for all 67 mothers and 64 newborns with HPRT who had the relevant biomarkers.

age, smoking status, dietary PAH intake; but there was greater use of coal for home heating in Limanowa (46.4% *versus* 25.6%; $P = 0.08$) and lower SES in Limanowa (college education as a proxy; $P = 0.005$). Daily ambient monitoring data were available only for Krakow. Monitoring data for Krakow for 1991–1992 were provided by the Division of National Sanitary Inspection (15 monitoring stations) and by the United States Environmental Protection Agency (five monitoring stations). Each Krakow woman's exposure to ambient particulates <10 μm in diameter (PM_{10}) was estimated by taking the average of PM_{10} measurements (in $\mu g/m^3$) reported at the monitoring station closest to her residence for the year before her delivery date. On the basis of these data, we estimate that in 1991, the year preceding the birth of the newborns, the Krakow women 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 (16). The corresponding concentrations of benzo(*a*)pyrene, often used as an indicator of PAH, were estimated to be 7 ng/m^3 and 15 ng/m^3. These benzo(*a*)pyrene concentrations were about 2–5-fold higher than in most Western countries.

Data Collection and Analysis of Biomarkers. In interviews administered after delivery using a standardized questionnaire, subjects provided information on demographic and environmental variables including age, active and passive cigarette smoking, diet (exposure to PAH/aromatics from broiled and smoked foods), and occupation (17). Coded samples of maternal peripheral blood (at least 30 ml) and umbilical cord blood were collected at delivery (20–60 ml) and were processed and stored as described (17). All laboratory personnel were blinded to subject identity.

Aromatic-DNA Adducts. The ^{32}P-postlabeling TLC assay was carried out on 15–20 μg of DNA as described (18). Adducts were enriched by nuclease P1 treatment. Two to five assays were carried out for each sample, and the mean of all assay results was calculated. The detection limit of the assay is 0.07 adducts/10^8 nucleotides. The method provides a summary measure of a complex mixture of bulky, hydrophobic adducts including aromatic adducts resistant to nuclease P1 digestion (19). In general, the ^{32}P-postlabeling method with nuclease P1 digestion is efficient for most PAH adducts but not for many aromatic amine adducts (20).

PAH-DNA Adducts. PAH-DNA adducts were measured by a competitive ELISA with fluorescence endpoint detection essentially as described previously (21). The detection limit of the assay is 2 adducts/10^8 nucleotides. Samples were assayed in triplicate at 50 μg of DNA/well (total, 150 μg DNA); the median values were used to determine the percentage of inhibition. When sufficient DNA was available (63% of samples), the assay was repeated. The antiserum recognizes structurally related PAH diol-epoxide-DNA adducts, including those formed by benzo(*a*)pyrene, benz[*a*]anthracene, and chrysene (22).

Plasma Cotinine. Liquid/liquid extraction of plasma was followed by gas chromatographic separation as described previously (17). An internal standard, *N*-methyl cotinine, was added to the plasma before extraction. The estimated half-life of cotinine in smokers is 21–30 h (23). In the chronic exposure situation, this marker is a good reflection of the daily uptake of cotinine (24).

***HPRT*.** The *HPRT* T-cell cloning assay was used to determine the frequency of cells that carry *HPRT* inactivating mutations as described previously (14, 25). Lymphocytes from ~10 ml of blood were required for this assay. Immediately after collection, the mononuclear cells were isolated from peripheral blood samples through density sedimentation on site in Poland and cryopreserved in medium containing 42% fetal bovine serum and 8% DMSO. After transport to the University of Vermont, the cells were thawed, cell number was determined, and cells were plated for the T-cell cloning assay. Previous studies have shown that fresh and frozen cells give similar MF results (26). *HPRT* MF is the ratio of cloning efficiencies in the presence and absence of TG. Maternal MF was not adjusted for maternal age because the two variables were not associated in this relatively homogeneously aged population. Because of missing or inadequate samples, results were available for 64 newborns and 67 mothers, of whom 48 were paired.

Statistical Analysis. Linear regression was used for continuous variables. *HPRT* MF data were natural-log transformed to achieve a normal distribution. Adducts and cotinine were treated as dichotomous variables (stratifying at the median). The mean biomarker levels in paired maternal and newborn samples were compared by paired *t* test (2-tailed, $\alpha = 0.05$). Because the mean levels of aromatic adducts and *HPRT* were higher in Limanowa than Krakow newborns ($P < 0.05$), regression analyses were run separately for the two groups. The effects of adducts on *HPRT* were similar in both groups; therefore, analyses were performed on the combined dataset. Adjustment of MF for nonselected cloning efficiency or removal of outliers did not materially alter the associations. Neither smoking (self-reported or maternal cotinine), residential coal

Table 2 Effects of DNA adducts on *HPRT* mutant frequency

DNA adducts	β, P^b
Newborn aromatic-DNA adducts (^{32}P)	0.60, 0.02
Newborn PAH-DNA adducts (ELISA)	0.17, 0.42
Maternal aromatic-DNA adducts (^{32}P)	0.09, 0.72
Maternal PAH-DNA adducts (ELISA)	0.07, 0.78

[a] The dependent variables are newborn *HPRT* MF for newborn adducts and maternal *HPRT* MF for maternal adducts. Each model was run separately.
[b] After adjustment for cotinine, diet, and SES, $\beta = 0.56$, $P = 0.03$.

use, SES, nor diet was a significant independent predictor of *HPRT* MF ($P \leq 0.05$). However, to distinguish the contribution of ambient pollution from that of other sources of the same pollutants, we included as covariates maternal cotinine and diet. SES (mother's education as a proxy) was also included as a covariate because it has been shown previously to be a significant determinant of MF (14). The regression analyses were run on 64 newborns and 67 mothers.

Results

In this cross-sectional study of 67 mothers and 64 newborns (48 pairs), plasma cotinine was significantly higher in newborns than mothers; and elevated levels of PAH-DNA and aromatic-DNA adducts in the newborns suggest differential susceptibility of the fetus to genetic damage (Table 1). Self-reported smoking, coal use, and diet were not significantly correlated with *HPRT* MF. However, among the newborns, *HPRT* was positively associated with aromatic adducts ($\beta = 0.60$, $P = 0.02$; Table 2). After adjustment for cotinine, diet, and SES, the association remained strong ($\beta = 0.56$, $P = 0.03$). Addition of coal use to the model did not alter the effect ($\beta = 0.56$, $P = 0.04$). These relationships were not observed among the mothers. Although the two adduct measures were correlated (Pearson's correlation = 0.30; $P = 0.01$), the more specific measure of DNA damage, PAH-DNA adducts, was not a significant predictor of *HPRT* MF ($\beta = 0.17$, $P = 0.42$). Although both methods detect PAH, the identity of the additional DNA adducts that differentiate the two assays remains unknown. There was no effect of self-reported smoking status of the mother during pregnancy (active, passive, or nonsmoker) on *HPRT* MF in either mothers or newborns. Maternal cotinine was not associated with *HPRT* MF in the mother or newborn.

Discussion

This study provides evidence that the fetus is especially sensitive to DNA damage from air pollution and that this damage can induce somatic mutation *in utero*. The observation of a significant effect of adducts on *HPRT* MF in newborns and not in mothers is consistent with the fetus being, in a sense, a "clean slate." Adducts have a half-life on the order of 16 weeks (27), and MF levels measured at birth predominantly reflect exposures during the past 3 months of prenatal development, the time of active T-lymphocyte proliferation and maturation. Thus, in the fetus both biomarkers reflect the same period of exposure, which is not the case in the mother, where MF in T cells may have accumulated over many years. There have been no previous studies of adducts and *HPRT* in the fetus, but among adults, correlations between aromatic DNA adducts and *HPRT* MF have been seen previously only in workers with heavy, continuous, long-term exposure (28, 29) and in smoking lung cancer cases (30). Neither smoking status nor measured cotinine was related to MF, consistent with a report that *HPRT* MF was not elevated in 12 infants whose mothers reported being exposed to secondhand smoke, compared with the same number of newborns without this exposure. However, in that study there was a significant difference in the *HPRT* mutational spectrum in the exposed infants (12). In related research, investigators from the Czech Republic have reported an association between placental DNA adducts and air pollution exposure (31). In conclusion, the finding that aromatic-DNA adducts predict MF is relevant to risk of cancer (7, 8, 32). The study provides molecular evidence that *in utero* exposure to environmental pollution may have serious health impacts.

Acknowledgments

We gratefully acknowledge the technical assistance of Amy Lafayette and Jesse Campbell on the *HPRT* mutation assay, Tie Lan Young for analysis of PAH-DNA, and K. Yang for postlabeling analysis.

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