Short Communication

In Utero DNA Damage from Environmental Pollution Is Associated with Somatic Gene Mutation in Newborns1

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 32P-postlabeling are positively associated with HPRT mutation frequency in the newborns (β = 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 ≤ 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 HPRT1 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 phosphorylates 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 106 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

1 The abbreviations used are: HPRT, hypoxanthine-guanine phosphoribosyltransferase; TG, 6-thioguanine; MF, mutation frequency; PAH, polycyclic aromatic hydrocarbon; SES, socioeconomic status.
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 32P-postlabeling TLC assay was carried out on 15–20 μg of DNA as described (18). Adducts were enriched by nucleic 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 32P-postlabeling method with nucleic 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, α = 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 1  Results for four biomarkers among the 48 paired Polish mothers and newborns with HPRT

<table>
<thead>
<tr>
<th>Exposure/Biomarker</th>
<th>Mothers</th>
<th>Newborns</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SE)</td>
<td>Mean (SE)</td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Plasma cotinine (ng/ml)</td>
<td>12.1 (5.0)</td>
<td>17.9 (5.9)</td>
</tr>
<tr>
<td>High</td>
<td>19 (39.6)</td>
<td>22 (45.8)</td>
</tr>
<tr>
<td>Low</td>
<td>29 (60.4)</td>
<td>26 (54.2)</td>
</tr>
<tr>
<td>PAH-DNA adducts (per 10^8 nucleotides)</td>
<td>6.1 (1.4)</td>
<td>10.0 (1.8)</td>
</tr>
<tr>
<td>High</td>
<td>22 (55.0)</td>
<td>23 (52.3)</td>
</tr>
<tr>
<td>Low</td>
<td>18 (45.0)</td>
<td>21 (47.7)</td>
</tr>
<tr>
<td>Aromatic-DNA adducts (per 10^9 nucleotides)</td>
<td>15.0 (2.1)</td>
<td>19.3 (1.9)</td>
</tr>
<tr>
<td>High</td>
<td>22 (50.0)</td>
<td>23 (50.0)</td>
</tr>
<tr>
<td>Low</td>
<td>18 (45.0)</td>
<td>21 (47.7)</td>
</tr>
<tr>
<td>HPRT MF (per 10^6 cells)</td>
<td>18.8 (2.7)</td>
<td>10.4 (5.6)</td>
</tr>
</tbody>
</table>

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 32P 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.
Table 2  Effects of DNA adducts on HPRT mutant frequency

<table>
<thead>
<tr>
<th>DNA adducts</th>
<th>β</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn aromatic-DNA adducts (1P)</td>
<td>0.60</td>
<td>0.02</td>
</tr>
<tr>
<td>Newborn PAH-DNA adducts (ELISA)</td>
<td>0.17</td>
<td>0.42</td>
</tr>
<tr>
<td>Maternal aromatic-DNA adducts (2P)</td>
<td>0.09</td>
<td>0.72</td>
</tr>
<tr>
<td>Maternal PAH-DNA adducts (ELISA)</td>
<td>0.07</td>
<td>0.78</td>
</tr>
</tbody>
</table>

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, β = 0.56, P = 0.03.

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.

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References


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