Transplacental Transfer of Environmental Genotoxins—Polycyclic Aromatic Hydrocarbon–Albumin in Nonsmoking Women

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Transplacental transfer of genotoxic material has been determined by measuring the polycyclic aromatic hydrocarbon–albumin adduct level in serum isolated from the mother and the umbilical cord blood using a competitive enzyme-linked immunoadsorbent assay (ELISA) and the antibody (8E11) against benzo[*a*]pyrene (B[*a*]P) tetrols. Smoking women (median = 5.54 fmol B[*a*]P Eq/μg albumin; $n = 21$ cases) and nonsmoking women living in rural areas (median = 4.99; $n = 30$) had higher adduct levels than nonsmoking women living in suburbia (median = 4.09; $n = 37$), whereas nonsmoking women living in the city of Aarhus had an intermediate level (median = 4.82; $n = 40$). The median adduct level in umbilical cord blood was significantly lower than in maternal blood, the maternal/fetal ratio being approximately 1.3. A positive association between the adduct levels in the mother and umbilical cord blood was observed. This study indicates that the competitive ELISA to detect B[*a*]P bound to serum albumin is sensitive enough to detect differences in the burden of genotoxic compounds in nonoccupationally exposed individuals. The lower adduct level in people living in suburbia suggests that local production of incomplete combustion products, like vehicle exhaust or heat generation, is a contributing factor of genotoxic compounds in the general environment. — Environ Health Perspect 104(Suppl 3):625–627 (1996)

Key words: biomarker, PAH exposure, albumin, human, placenta, general environment

Introduction

Transplacental exposure to carcinogens has been proven to induce tumors in the offspring of experimental animals treated with high doses of carcinogens (*1*). In humans, a potential association between parental occupational exposure and increased risk of cancer in offspring has been established (*2*), while no consistent relationship between maternal smoking during pregnancy and childhood cancer risk could be established (*3*). Transplacental transfer of genotoxins in humans has been demonstrated by the presence of carcinogen–macromolecule adducts in either umbilical cord blood proteins or DNA isolated from fetal tissues. Hemoglobin adducts, formed by hydroxyethylating agents (*4*) and 4-aminobiphenyl (*5*) have been detected in the blood of newborns from both smoking and nonsmoking mothers, while transplacental transfer of polycyclic aromatic hydrocarbons (PAHs) has been demonstrated by the formation of PAH–albumin adducts (*6*). The ^{32}P-postlabeling assay has been used to demonstrate the presence of bulky carcinogen–DNA adducts in fetal tissues, i.e., liver, lung, and umbilical cord from both smoking and nonsmoking mothers (*7,8*). A large interindividual variation in the adduct levels was observed, which could be a reflection of different levels and sources of exposure, as well as a variation in the ability to biotransform the genotoxic compounds.

In this study, the burden of genotoxic compounds and transplacental transfer of one of these compounds present in cigarette smoke and ambient air has been investigated by determination of PAH–albumin adduct levels in both maternal and umbilical cord blood, and the effect of the glutathione *S*-transferase M1 (*GSTM1*) genotype on adduct level in nonsmoking mothers has been assessed.

Subjects and Methods

Population

The study cohort consisted of 130 pregnant women 19 to 44 years of age (mean = 29) living in the county of Aarhus, which includes both rural, urban, and suburban areas. The cohort was divided into four groups according to smoking habits or residence, the latter being based upon the postal code: group 1, nonsmoking with residence in the city center of Aarhus ($n = 40$); group 2, nonsmoking with residence in the suburban part ($n = 37$); group 3, nonsmoking with residence in the rural areas ($n = 30$); and group 4, smokers ($n = 21$) and occasional smokers ($n = 2$) living in Aarhus County. None of the women were occupationally exposed to known genotoxic compounds prior to pregnancy.

All cases were collected in the period between November 1993 and January 1994 at the Department of Gynecology/Obstetrics at Aarhus University Hospital. Prior to the collection, all the women gave informed consent according to the Helsinki II declaration.

A questionnaire to assess the potential exposure to genotoxic compounds was administered 2 months before delivery. Information on residence, occupation, means of transportation, and lifestyle factors, including passive smoking, were collected. Nonsmoking status was verified by determination of cotinine in serum samples using the cotinine EIA microplate assay (Solar Care Technologies Corporation, Bethlehem, PA).

Analysis of Blood Samples

Maternal and cord blood (5 ml) were collected in dry tubes and serum was

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Abbreviations used: ELISA, enzyme-linked immunoadsorbent assay; B[*a*]P, benzo[*a*]pyrene; BPDE, benzo[*a*]pyrene diol epoxide; PAH, polycyclic aromatic hydrocarbon.

isolated. Two milliliters of whole blood was collected in heparin tubes for DNA isolation. The maternal blood sample was collected shortly after delivery.

Albumin was precipitated from serum, and the level of B[*a*]P bound to serum albumin was determined by a competitive enzyme-linked immunoadsorbent assay (ELISA) as previously described (*6*).

The competitive ELISA was performed in polystyrene 96-well plates (NUNC immuno plates; Nunc, Roskilde, Denmark) using benzo[*a*]pyrene diol epoxide (BPDE)-modified dextran-78500 (equivalent to 750 ng BPDE/well). Control wells were coated with unmodified dextran. B[*a*]P tetrols were used as the standard (1–500 nM). The level of adducts was estimated from the standard inhibition curve and expressed as fmol B[*a*]P Eq/µg serum albumin. The lower level of detectability was 10 nM B[*a*]P tetrols. Only the linear range of the inhibition curve was used for quantification. The same control serum sample was included on each plate to adjust for interplate and day–day variation.

Determination of *GSTM1* Genotype

The maternal *GSTM1* was determined by a slight modification of the procedure described by Zhong et al. (*9*) using DNA isolated from whole blood.

Statistical Analysis

The Mann-Whitney 2-tailed test was used to compare the adduct levels in the different groups and to evaluate associations between adduct levels and the potential source of exposure. The Kruskal-Wallis test was used to evaluate the effect of the *GSTM1* genotype on the adduct level on all maternal groups combined and the Pearson χ^2 test in the subgroups.

Results

The PAH–serum albumin adduct was detectable in all collected samples. The adduct level showed a large interindividual variation with a range of 1.79 to 13.89 fmol/µg albumin (median = 4.67). The adduct level was slightly higher in the smokers and occasional smokers group (median = 5.78) than in the nonsmoking group (median = 4.67; $p = 0.0454$ by the Mann-Whitney 2-tailed test). Among the nonsmoking women (Figure 1), those living in the suburban area had a significantly lower adduct level than the women living in the city ($p = 0.0173$), whereas the adduct level in women living in the rural areas was not significantly different from that in women in the city ($p = 0.9432$).

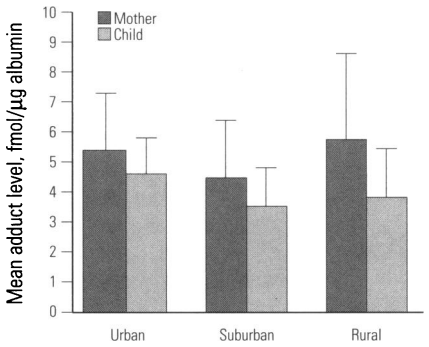

Figure 1. PAH–albumin adduct levels in maternal blood samples collected in the county of Aarhus. The residential classification is based upon postal codes. The results are expressed as mean ± SEM.

The serum albumin adduct levels in the combined nonsmoker groups were analyzed with respect to the information obtained from questionnaires (Table 1). Exposure to passive tobacco smoke did not significantly alter the adduct levels. Using a linear regression analysis, no association between the number of hours exposed to passive smoke and adduct levels could be observed ($R^2 = 0.013$; $p = 0.5122$). The type of diet and methods of preparing food, such as grilling and cooking, did not appear to influence the BPDE adduct level in maternal blood.

The adduct level in the umbilical cord blood was significantly lower than in the maternal blood in all residential groups ($p < 0.000$; $n = 104$). A positive association could be observed in the adduct levels in umbilical cord blood and maternal blood ($R^2 = 0.33356$; $p < 0.000$). The maternal/fetal ratio in adduct levels was slightly higher in smokers and rural residents (median ratio = 1.4) than in nonsmoking residents in the city and suburban areas (median ratio = 1.2).

The frequency of the *GSTM1* null genotype in the study population was 56%. The maternal *GSTM1* genotype did not significantly influence the PAH–albumin adduct level in either maternal or umbilical cord blood when all groups were combined (Kruskal-Wallis test; $p = 0.5148$), and the *GSTM1* genotype did not have any effect on the binding levels in subsets, e.g., smokers and city dwellers.

Table 1. Potential contributing factors for PAH–serum albumin adduct levels.

Parameter (number)	*p* Values
Smoking (21) vs nonsmoking (107)	0.0454
Nonsmokers (107)	
City (40) vs suburban (37)	0.0173
City (40) vs rural (30)	0.9432
Rural (30) vs suburban (37)	0.0561
Passive smoking	
Regularly in contact with smokers for 1 or more hours; yes (49) vs no (54)	0.7790
Transportation time, nonsmokers	
0–20 min (53) vs 21–240 min (41)	0.0537

Discussion

Human exposure to genotoxic compounds has been assessed using different types of biomarkers, but most of these studies have been in occupational cohorts with high levels of exposure (*10*). Serum albumin adducts have been used to assess the exposure to PAH (*6,11,12*) and aflatoxin (*13*) in both the general population and in occupationally exposed individuals. The advantage of using binding to blood protein as a marker for exposure is the availability of biological material and the fact that the adduct level represents accumulated exposure within the half-life of the protein.

B[*a*]P is ubiquitous in the general environment; however, there is limited information on the exposure to carcinogenic PAHs in general populations. In this study, exposure to B[*a*]P or other PAHs, recognized by the monoclonal antibody against BPDE (*12*), could be detected in young women not occupationally exposed to PAHs. The PAH–albumin adduct level in maternal blood was lower than in people occupationally exposed to PAHs (*14*). The adduct level was only slightly higher in smokers than in nonsmoking city residents, which may be due in part to the number and type of cigarettes smoked by pregnant women in Denmark.

Potential sources for PAHs in the general environment are ingestion of PAH-contaminated food items (*15*), traffic-generated air pollution, and combustion products from energy production including residential heating (*16*). The women with the highest adduct levels in the urban group were predominantly living close to road intersections with high traffic density. The higher mean and median adduct levels in women living in rural areas is puzzling and cannot be explained on the basis of proximity to highly trafficked road or point sources of PAH pollution. A higher PAH–albumin adduct level has also been reported in young nonsmoking males living in rural areas than in an urban group (*17*). A higher ethylene oxide–valine

adduct level has also been reported in the rural group compared with the suburban group (*18*). Because the samples in these studies were collected in the months of November and December, incomplete combustion of energy sources used for domestic heating may be a contributing factor. In the city and in suburban areas, the source of heating is community based, whereas oil, wood, or straw are more frequently used as energy sources in rural areas. Straw is frequently used as a source of energy in utility plants in small communities. It has previously been shown that burning of wood and straw generates a high level of mutagenic active compounds, including different PAHs (*19*).

The placenta provides some protection against genotoxic compounds because the levels of adducts in cord blood were lower than those in the maternal blood in all groups. The maternal/fetal ratio was lower than the ratio calculated for the binding of bulky DNA adducts to maternal and fetal tissues (*8*) and for the transfer of ethylene oxide and 4-aminobiphenyl using binding to hemoglobin as a marker of transplacental transfer (*4,5*).

Most of the carcinogens present in cigarette smoke and in the general environment are biotransformed. Some of these metabolic processes are mediated by members of the cytochrome P450 family of enzymes or glutathione *S*-transferases. The multilocus glutathione *S*-transferase family of enzymes catalyzes the reaction between glutathione and, for example, the ultimate carcinogenic form of B[*a*]P, BPDE, hence preventing the binding to DNA and other macromolecules. In this study, the *GSTM1* genotype did not have any significant effect on the PAH–albumin adduct level in the subgroups, and trend analysis did not reveal any association between genotype and adduct level. The level of exposure to B[*a*]P in the present study is low, and alternative detoxification pathways may be in operation.

This study indicates that biomarkers for genotoxic exposure can be used on nonoccupationally exposed and nonsmoking people. The source of the genotoxic material appears to be traffic-generated pollution or products formed by incomplete combustion in residential heating systems. The presence of adducts in cord blood is an indirect sign of genotoxic damage to the fetus, and subsequently, the initiation of a cancer cell may have occurred *in utero,* resulting in an increased risk or earlier occurrence of cancer in adult life. However, there is no epidemiological data to suggest that this low level of genotoxic exposure, in suburban areas for example, is associated with a lower cancer risk.

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