Mutation Research 669 (2009) 13–19

Contents lists available at ScienceDirect

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis

journal homepage: www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

Biomarkers of exposure to tobacco smoke and environmental pollutants in mothers and their transplacental transfer to the foetus. Part I: Bulky DNA adducts

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ARTICLE INFO

Article history:
Received 6 January 2009
Received in revised form 21 April 2009
Accepted 24 April 2009
Available online 9 May 2009

Keywords:
Air pollution
DNA adducts
PAHs
Complex mixtures
Placenta
Tobacco smoke

ABSTRACT

^{32}P-postlabelling and PAH-ELISA using the antiserum #29 were employed to analyze DNA adducts in venous and umbilical cord blood and the placenta of 79 mothers giving birth to 80 living babies in Prague (Czech Republic). Ambient air exposure was measured by stationary measurements of basic air pollutants (PM2.5, c-PAHs) during the entire pregnancy. Tobacco smoke exposure was assessed by questionnaire data and by plasma cotinine levels. The total DNA adduct levels in the lymphocytes of mothers and newborns were elevated by 30–40% ($p < 0.001$) compared with the placenta. B[a]P-like DNA adduct (adduct with the identical chromatographic mobility on TLC as major BPDE derived DNA adduct) levels were elevated in the blood of mothers compared with the placenta and the blood of newborns ($p < 0.05$ and $p < 0.01$). In tobacco smoke-exposed mothers, higher DNA adduct levels in the blood of mothers and newborns compared with the placenta were found ($p < 0.001$), whereas the total and B[a]P-like adduct levels were comparable in the blood of mothers and newborns. B[a]P-like adducts were elevated in the blood of mothers unexposed to tobacco smoke compared with that of corresponding newborns and the placenta ($p < 0.01$). Total and B[a]P-like DNA adducts were increased in the placenta of tobacco smoke-exposed compared with unexposed mothers ($p < 0.001$ and $p < 0.01$). In lymphocytes of tobacco smoke-exposed mothers, the comparison of total adduct levels (1.18 ± 0.67 vs. 0.92 ± 0.28) and B[a]P-like DNA adducts (0.22 ± 0.12 adducts/10^8 nucleotides vs. 0.15 ± 0.06 adducts/10^8 nucleotides) with newborns indicated a 30–40% increase of adducts in mothers. Almost equal PAH–DNA adduct levels were detected by anti-BPDE-DNA ELISA in the placenta of tobacco smoke-exposed and -unexposed mothers. Our results suggest a protective effect of the placental barrier against the genotoxic effect of some tobacco smoke components between the circulation of mother and child. We found a correlation between adduct levels in the blood of mothers and newborns.

1. Introduction

Ambient air pollution, caused in part by environmental tobacco smoke (ETS) and carcinogenic polycyclic aromatic hydrocarbons (PAHs), has been studied extensively in relation to various aspects of human and particularly child health. This is because of the growing evidence that foetuses and children are more sensitive than adults to the toxicity of many environmental air pollutants because of their higher proliferation rate, lower immunologic competence and reduced ability to detoxify carcinogens and repair DNA damage [1–7]. The placenta serves as an intermediary between maternal and foetal circulations. It is not merely a passive barrier between them, but has many physiological functions, including the exchange of respiratory gases, metabolites, nutrients and waste products, as well as the production of hormones and the metabolism of xenobiotics [8–9]. The placenta is readily accessible and responsive to environmental pollutants such as PAHs [10]. The ability of PAHs to reach placental tissue and cord blood

Abbreviations: B[a]P, benzo[a]pyrene; B[a]P-like adduct, DNA adduct with the identical chromatographic mobility on TLC as major BPDE derived adduct; B[b]F, benzo[b]fluoranthene; B[k]F, benzo[k]fluoranthene; B[a]A, benz[a]anthracene; B[ghi]P, benzo[ghi]perylene; BPDE, (*7R,8S*)-dihydroxy-(*9S,10R*)-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; BPDE-N^2-dG, N^2-[7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene-10-yl]deoxyguanosine; c-PAHs, carcinogenic polycyclic aromatic hydrocarbons (include benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene, chrysene, dibenz[ah]-anthracene, and indeno[cd]pyrene); CHRY, chrysene; DRZ, diagonal radioactive zone on TLC chromatogram; DB[al]P, dibenzo[al]pyrene; DB[ah]A, dibenz[ah]anthracene; DCM, dichlormethane; 7,12-DMBA, 7,12-dimethylbenz[a]anthracene; HPLC, high performance liquid chromatography; I[cd]P, indeno[cd]pyrene; PM2.5, particles having aerodynamic diameter 2.5 μm and smaller; RAL, relative adduct labelling; SDS, sodium dodecyl sulphate; TLC, thin layer chromatography.

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doi:10.1016/j.mrfmmm.2009.04.011

has been reported in few studies [11–12]. A positive association between cigarette smoking during pregnancy and DNA adduct levels detected by ^{32}P-postlabelling in the placenta was repeatedly observed [13–14]. Perera et al. [15,16] demonstrated that newborns with elevated PAH–DNA adducts and hypoxanthine-guanine phosphoribosyltransferase (HPRT) mutations in cord blood had significantly reduced birth weight, length and head circumference compared with newborns with lower cord blood DNA adduct levels and HPRT mutations.

A recent study from India [17] reported high levels of PAHs in the placenta of women heavily exposed to PAHs. The study indicated an association between the risk of preterm delivery and the levels of some PAHs in placenta. Exposure to cigarette smoke during pregnancy is deleterious to foetal development as reflected by reduced neonatal weight at birth [18]. Placental weight reduction is indistinct, but the placenta expressed markedly augmented overall xenobiotic (PAHs) metabolism capability in response to cigarette smoke exposure during pregnancy, indicating that placental metabolism may be an important mediator of adverse effects induced by exposure to tobacco smoke [19].

To study the potential transfer of genotoxic compounds bound on particulate matter (PM) such as PAHs in ambient air and in the tobacco smoke from the exposed mothers to the foetus, bulky DNA adduct levels have been measured by ^{32}P-postlabelling in the venous blood of mothers, in the placenta and in umbilical cord blood. In the placenta PAH–DNA adducts have also been analyzed by enzyme-linked immunosorbent assay (ELISA) using the antibody raised against benzo[a]pyrene-r-7,t-8-dihydrodiol-t-9,10-epoxide [±] (BPDE)-modified DNA. Simultaneously, the biomarkers of the oxidative damage of biological macromolecules (DNA, proteins, lipids) have been studied in the same subjects and are reported in part II of this study [20].

2. Materials and methods

2.1. Chemicals

Spleen phosphodiesterase was purchased from ICN Biomedicals, ribonuclease A and T1, proteinase K, micrococcal nuclease and protein assay kit (Number 5656) from Sigma (Deisenhofen, Germany); polyethylene-imine-cellulose TLC plates (0.1 mm) from Macherey-Nagel (Düren, Germany); nuclease P1 (Japan Institute for the Control of Aging, Japan); T4 polynucleotide kinase (USB, Germany); γ-^{32}P-ATP (3000 Ci/mmol, 10 μCi/μl) from Amersham Biosciences. The radioimmunoassay kit was obtained from Brandeis University (USA). All other chemicals and solvents were of HPLC or analytical grade.

2.2. Subjects and sampling

Each woman involved in the study gave birth to a living baby in the University Hospital in Prague between 17 June and 26 June 2007 (12 deliveries) and 8 October 2007 and 26 February 2008 (67 deliveries). Information on the course and outcome of the pregnancy was obtained from medical records. Informed consent was obtained from each mother participating in this study. Mothers (N = 79) were interviewed by the physician who administered the questionnaire concerning lifestyle. Mothers were aged 30.2 ± 4.7 years [median (range): 31 (20–44) years]. Newborns (N = 80) had birth weight 3390 ± 420 g [median (range): 3450 (2020–4130) g]. Samples of the maternal venous blood and umbilical cord blood collected into EDTA-containing vacutainer tubes (Greiner Bio-One, Austria) were centrifuged for 10 min at 2000 rpm at room temperature. Aliquots of plasma were taken and frozen for the analysis of cotinine, vitamins A and E, and the oxidative damage of some macromolecules reported in part II of this study [20]. The rest of the samples were used for lymphocyte isolation. Sampling of placenta tissue was done as previously described [21]. The villus parenchyma sections were obtained by dissecting a 1.5-cm cubic-shaped segment (approximately 5-cm away from the site of cord insertion) and then splitting it into three equal parts: maternal (including thin basal plate), middle, and foetal (including the chorionic plate). The middle sections were frozen and stored at −80 °C until DNA isolation.

2.3. Monitoring of air pollution

Stationary measurements of basic air pollutants—PM2.5 (particles having aerodynamic diameter 2.5 μm and smaller) and c-PAHs were done to assess the level of ambient air pollution and the external exposure of subjects. Data from stationary measuring stations in Prague 5-Smíchov were used. The daily concentrations of air pollutants including c-PAHs in separate periods of the study were monitored using versatile air pollution samplers (VAPS). c-PAHs include benzo[a]pyrene (B[a]P), benz[a]anthracene (B[a]A), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo[ghi]perylene (B[ghi]Pe), chrysene (CHRY), dibenz[ah]anthracene (DB[ah]A), and indeno[cd]pyrene (I[cd]P).

2.4. DNA isolation

Lymphocytes were isolated from whole blood using Ficoll 400 gradient centrifugation. Cell pellets were homogenised in a solution of 10 mM Tris–HCl, 100 mM EDTA and 0.5% sodium dodecyl sulfate (SDS), pH 8.0. DNA was isolated using RNAses A and T1 and proteinase K treatment followed by phenol/chloroform/isoamyl alcohol as previously described [22]. DNA concentration was estimated spectrophotometrically by measuring ultraviolet (UV) absorbance at 260 nm. DNA samples were stored at −80 °C until analysis.

2.5. DNA adducts by ^{32}P-postlabelling

^{32}P-postlabelling analysis of bulky DNA adducts in lymphocytes and placenta was done as previously described [23–24]. Briefly, DNA samples (6 μg) were digested by a mixture of micrococcal endonuclease and spleen phosphodiesterase for 4 h at 37 °C. Nuclease P1 was used for adduct enrichment. Labelled DNA adducts were resolved by two-directional thin layer chromatography on 10 cm × 10 cm polyethylene-imine (PEI)-cellulose plates. Solvent systems used for thin layer chromatography (TLC) were: D-1: 1 M sodium phosphate, pH 6.8; D-2: 3.8 M lithium formate, 8.5 M urea, pH 3.5; D-3: 0.8 M lithium chloride, 0.5 M Tris, 8.5 M urea, pH 8.0. Autoradiography was carried out at −80 °C for at least 72 h. Total DNA adduct levels were evaluated from the diagonal radioactive zones (DRZ) on thin layer chromatograms. The DRZ represents the mixture of many overlapping DNA adduct spots originating from the various adduct forming substances in the environment including tobacco smoke. B[a]P-like DNA adducts were determined using radioactivity detected in the area of chromatograms corresponding to the major B[a]P-derived DNA adduct N^2-[7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene-10-yl]deoxyguanosine (BPDE-N^2-dG). The name B[a]P-like adduct reflects the fact that this adduct is the mixture of various adducts induced by the exposure to complex mixture of genotoxic compounds such as c-PAHs. The radioactivity of distinct adduct spots was measured by liquid scintillation counting. To determine the exact amount of DNA in each sample, aliquots of the DNA enzymatic digest (1 μg of DNA hydrolysate) were analyzed for nucleotide content by reverse-phase high performance liquid chromatography (HPLC) with UV detection, which simultaneously allowed for controlling the purity of the DNA. DNA adduct levels were expressed as adducts per 10^8 nucleotides. A major B[a]P-derived adduct (BPDE-N^2-dG) obtained after oral administration of B[a]P 100 mg/kg body weight of rats (DNA from liver tissue) was a positive DNA control and was analyzed in each experiment to check variability between experiments.

2.6. DNA adducts by ELISA

PAH–DNA adducts were analyzed in the placenta by competitive ELISA essentially as previously described [25] with some modifications. The antiserum #29 used for the detection of PAH–DNA adducts was raised in New Zealand White rabbits against BPDE-modified calf thymus DNA [26]. The antiserum was kindly provided by Professor Regina Santella, Columbia University, NY, USA.

BPDE-modified DNA was prepared by the incubation of 4.5 mg BPDE with 10 mg calf thymus DNA at room temperature overnight followed by the extraction with water-saturated diethyl ether and isoamyl alcohol. The level modification of the BPDE-DNA was 78 pmol/μg DNA. The modified DNA was used for coating ELISA plates, as well as for preparation of standard curves. The limit of detection of the assay was three PAH–DNA adducts/10^8 nucleotides; the 50% inhibition of standard curves ranged from 28 to 34 fmol/μg DNA. Wells of ELISA plates were coated with 10 ng of BPDE-DNA (total volume, 50 μl/well) by drying the plates overnight at room temperature. Plates were washed with PBS/Tween (0.05% Tween 20 in phosphate-buffered saline (PBS)) and blocked with 200 μl/well of blocking buffer (1% foetal calf serum (FCS) in PBS/Tween) for 1 h at room temperature. After blocking, 50 μl of BPDE-DNA standards (concentration range, 4.5–144 fmol/μg DNA) and DNA samples (concentration range, 0.75–1.25 mg/ml, total amount 37.5–62.5 μg of DNA/well) were added, followed by 50 μl of primary antibody (antiserum # 29; final dilution 1:1,000,000). After incubation for 1.5 h at 37 °C and washing, 100 μl of secondary antibody conjugated with alkaline phosphatase (Sigma) was added. Another 1.5 h incubation at room temperature was followed by washing with PBS/Tween and with 0.01% diethanolamine in water. The colour was developed by adding 100 μl of p-nitrophenyl phosphate substrate (1 mg/ml of 1 mol/l diethanolamine) per well and incubating the plates for 30–60 min at room temperature. Absorbance was measured with a microplate reader at 405 nm. Each sample was analyzed in triplicate. PAH–DNA levels were corrected per 1 μg of DNA and expressed as adducts/10^8 nucleotides.