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Environmental exposure to carcinogenic polycyclic aromatic hydrocarbons—The interpretation of cytogenetic analysis by FISH

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Abstract

The capital city of Prague is one of the most polluted localities of the Czech Republic. The effect of exposure to carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) adsorbed onto respirable air particles (<2.5 μm) on chromosomal aberrations was studied in a group of city policemen (street patrol, aged 34 ± 8 years) working in the downtown area of Prague and spending daily >8 h outdoors ($N = 61$) in months of January and March 2004. Ambient air particles (PM10, PM2.5) and c-PAHs were monitored using Versatile Air Pollution Sampler (VAPS), and personal exposure was evaluated using personal samplers during working shift. Chromosomal aberrations were analyzed by fluorescent in situ hybridization (FISH) and conventional cytogenetic analysis. Urinary cotinine, plasma levels of vitamins A, E and C, folate, total cholesterol, HDL, LDL cholesterols and triglycerides were also analyzed as possible effect modifiers. During the sampling period the particulate air pollution monitored by VAPS was in January versus March as follows: PM10 55.6 μg/m^3 versus 36.4 μg/m^3, PM2.5 44.4 μg/m^3 versus 24.8 μg/m^3, c-PAHs 19.7 ng/m^3 versus 3.6 ng/m^3, and B[a]P 4.3 ng/m^3 versus 0.8 ng/m^3. Significant differences were observed for all FISH endpoints studied for the sampling in January and March (%AB.C. = 0.27 ± 0.18 versus 0.16 ± 0.17, $p < 0.001$, $F_G/100 = 1.32 \pm 1.07$ versus 0.85 ± 0.95, $p < 0.01$, AB/1000 (aberrations/1000 cells) = 4.27 ± 3.09 versus 2.59 ± 2.79, $p < 0.001$) while conventional cytogenetic analysis did not reveal any differences in the frequency of chromosomal aberrations. Factors associated with an increased level of translocations by FISH indicated the effect of age, cholesterol, LDL-cholesterol and vitamin C. We may conclude that FISH indicates that the city policemen in Prague represent a group of increased genotoxic risk. This is the first study reporting that translocations induced by c-PAHs in peripheral lymphocytes last only several weeks.

Keywords: Chromosomal aberrations; Conventional cytogenetic analysis; Fluorescence in situ hybridization; Whole chromosome painting; Environmental exposure to carcinogenic polycyclic aromatic hydrocarbons

Abbreviations: c-PAHs, carcinogenic polycyclic aromatic hydrocarbons; B[a]P, benzo[a]pyrene; PM10, particulate matter < 10 μm; PM2.5, particulate matter < 2.5 μm; CCA, conventional cytogenetic analysis; FISH, fluorescence in situ hybridization; PBL, peripheral blood lymphocytes; %AB.C., percentage of aberrant cells

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1. Introduction

Cytogenetic analysis of peripheral blood lymphocytes (PBL) has been accepted as a technique suitable for the biological monitoring of genetic damage in somatic cells since the early 1970s. Up to the present time, it has remained the only suitable assay for biological monitoring of the genetic damage induced in somatic cells by excessive exposure to clastogenic agents in the workplace (Natarajan and Obe, 1980; Carrano and Natarajan, 1988; Albertini et al., 2000; Sram et al., 2004b). Today, chromosomal aberrations in human PBL are recognized as an integrated marker of clastogenic exposure and cancer susceptibility, and are the only biomarker of effect which has been internationally standardized and validated (Hagmar et al., 2004; Bonassi et al., 2005; Rössner et al., 2005; Norppa et al., 2006; Boffetta et al., 2007).

When the whole chromosome painting by fluorescence in situ hybridization (FISH) was introduced in the 1990s in the field of ionizing radiation research (Tucker et al., 1993), the classic cytogenetic analysis of chromosomal damage was supplanted by FISH (Tucker, 2001). In comparison with conventional cytogenetic analyses (CCA), which detect particularly unstable types of aberrations, FISH using whole chromosome painting was developed as a rapid and sensitive method of detecting structural rearrangements, especially reciprocal translocations (Stronati et al., 2001; Duran et al., 2002). The FISH technique detects translocations, which are long lasting injuries likely transferred through many cell cycles. These types of chromosomal changes may circulate in PBL for a long period of time and may be related to cancer.

While the FISH technique is highly suitable for analyzing a low-dose radiation exposure (Hsieh et al., 2001; Awa, 2003), available data are still scarce as to ability to detect effect of exposures to chemical carcinogens. However, the FISH painting technique appears to be more sensitive than the conventional technique to detect the genomic frequency of translocations induced by various chemical agents (Verdorfer et al., 2001); e.g. for occupational exposure to acrylonitrile, 1,3-butadiene, ethylbenzene (Sram et al., 2004a) and environmental exposure to carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) (Sram et al., 2007).

Atmospheric pollution by c-PAHs from incomplete combustion represents a relevant environmental hazard and has been associated with a considerable amount of adverse health effects in humans. This prompted a study on the ability of ambient air particulate pollution in Prague to induce chromosomal aberrations in city policemen and "unexposed" controls by two methods: conventional method and FISH. This same cohort of subjects was evaluated during the winter and found that ambient air pollution to c-PAHs during this period significantly increased the genomic frequency of translocations in the city policemen compared to controls, which was not observed by CCA (Sram et al., 2007). In this current study, we followed the same city policemen who were evaluated repeatedly in January and March, when the significant difference between the exposure to c-PAHs was observed.

2. Materials and methods

2.1. Subjects and sampling

The study population consisted of 61 city policemen (ambient exposure during the street patrol), nonsmokers working in the Prague downtown and spending daily >8 h outdoors. The questionnaires on personal medical history and life-style (smoking, alcohol consumption, eating habits) had to be filled in by all participants. In addition, analysis of cotinine level in urine and vitamins A, C, E, and folate, as well as lipids in plasma were done to obtain objective status for smoking and diet.

All participating subjects were healthy volunteers, who signed an informed consent form and could cancel their participation at any time during the study according to Helsinki II declaration. Any person with medical treatment, radiography or vaccination up to 3 months before sampling was not included in the study.

The blood and urine samples were collected at the end of working shift, by venipuncture into vacutainers containing sodium heparin, coded and transported to the laboratory. The urine samples were kept at −80 °C until cotinine analysis. Blood samples were processed within 24 h.

The sampling of this group was carried out in January and March 2004 when the highest air pollution levels were expected. Personal exposure monitoring using personal samplers for collection of $PM_{2.5}$ particles from ambient air was performed during whole working shift as previously described (Binkova et al., 1998). Quantitative chemical analysis of carcinogenic PAHs (c-PAHs), including benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene, benzo[a]pyrene, chrysene, dibenz[ah]-anthracene and indeno[cd]pyrene, was performed by HPLC with fluorimetric detection according to the EPA method in the laboratories of the certified company ALS Czech Republic, Prague (certification: EN ISO CSN IEC 17025). The personal monitoring was supplemented with daily air pollution data from two monitoring stations located in the downtown and suburban areas. During the sampling period the particulate air pollution monitored by VAPS was in January versus March as follows: PM10 55.6 versus 36.4 $\mu g/m^3$, PM2.5 44.4 versus 24.8 $\mu g/m^3$, c-PAHs 19.7 versus 3.6 ng/m^3, and B[a]P 4.3 versus 0.8 ng/m^3.