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Air pollution by carcinogenic PAHs and plasma levels of p53 and p21^{WAF1} proteins

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Abstract

We analyzed the effect of exposure to carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) in ambient air on the plasma levels of p53 and p21^{WAF1} proteins among city policemen, bus drivers and controls in three European cities: Prague (Czech Republic), Kosice (Slovakia) and Sofia (Bulgaria). p53 and p21^{WAF1} proteins are key regulators of the cell cycle and are accepted as universal markers of genotoxic stress and DNA damage. In total 204 exposed subjects (100 smokers, 104 nonsmokers) and 152 controls (54 smokers, 98 nonsmokers) were analyzed. Personal exposure to c-PAHs was evaluated using personal samplers during the working shift. The levels of p53 and p21^{WAF1} proteins were assessed by ELISA assay. There were no differences between the levels of either protein between exposed and controls, or smokers and nonsmokers, in any city. However, we observed significant differences in p53 plasma levels in all subjects regardless of the exposure status between the individual cities (median values: 5, 31, 234 pg/ml, $p < 0.001$, for Prague, Kosice and Sofia, respectively). The levels correspond to the differences in exposure levels to c-PAHs and benzo[*a*]pyrene (B[*a*]P) in the individual cities. A multiple linear regression analysis confirmed that c-PAHs exposure is a variable significantly affecting levels of both proteins in all locations. When all subjects were divided into the group exposed to below-median levels of c-PAHs and the group exposed to above-median levels of c-PAHs we found significantly higher p53, as well as p21^{WAF1} levels in the above-median exposure group (p53, 167 pg/ml versus 25 pg/ml, $p < 0.001$; p21^{WAF1}, 2690 pg/ml versus 2600 pg/ml, $p < 0.05$). Among all subjects p53 plasma levels were positively correlated with p21^{WAF1} levels, exposure to B[*a*]P, c-PAHs and levels of total DNA adducts; for p21^{WAF1} levels we observed the positive correlation with cotinine, c-PAHs exposure, total and B[*a*]P-like DNA adduct levels. In conclusion our results suggest that p53 and p21^{WAF1} proteins plasma levels may be useful biomarkers of c-PAHs environmental exposure.

Keywords: p53 and p21^{WAF1} plasma levels; Air pollution; B[*a*]P and c-PAHs exposure

1. Introduction

Respirable particulate matter of an aerodynamic diameter <10 μM (PM_{10}) poses a potential health hazard. Particulate matter consists of a complex mixture of chemicals, many of them have been shown to be toxic or potentially carcinogenic. Epidemiological studies demonstrated increased mortality and morbidity

Abbreviations: B[*a*]P, benzo[*a*]pyrene; c-PAHs, carcinogenic polycyclic aromatic hydrocarbons; PM_{10}, respirable particulate matter of an aerodynamic diameter <10 μM; TMB, tetramethyl benzidine

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of cardiovascular and respiratory diseases, as well as increased risk of cancer associated with exposure to genotoxic compounds in polluted ambient air [1–3]. c-PAHs adsorbed on particulate matter are believed to be one of the major sources of cancer risk. Health consequences of c-PAHs exposure were the point of interest of several molecular epidemiological studies [4]. Despite the improving air quality due to the reduction of industrial and heating emissions, the increasing number of motor vehicles in cities is the persisting problem with a potentially serious impact on the health of their inhabitants. The effort to monitor the impact of air pollution on human health in metropolitan areas resulted in the EC project, EXPAH [5]. As the part of the project, 204 city policemen or bus drivers and 152 control subjects from three European cities, Prague (Czech Republic), Kosice (Slovakia) and Sofia (Bulgaria), were monitored in the winter season, individual exposure levels were assessed and a number of biomarkers were analyzed, among them p53 and $p21^{WAF1}$ plasma levels [5].

p53 protein is a tumor suppressor that is induced after DNA damage by chemical mutagens, UV or γ-irradiation, and by oxidative stress. Its expression results either in cell cycle arrest or apoptosis [6]. Mutations in the *p*53 gene are the most common genetic changes in human cancers. $p21^{WAF1}$ protein is a cyclin-dependent kinase inhibitor induced by p53 protein. It arrests the cell cycle either in the G1, S or G2 phase [7]. It has been shown that the expression of both p53 and $p21^{WAF1}$ proteins is induced *in vitro* by c-PAHs [8,9].

p53 was suggested to be an unspecific biomarker of genotoxic stress. Blood serum or plasma levels of p53 were used as markers of occupational exposure to chromium [10], asbestos [11], PAHs [11–13], vinyl chloride [14], acrylonitrile [15], formaldehyde [16] and ionizing radiation [17,18]. There is some controversy regarding the analysis of p53 protein in biological fluids like blood serum or plasma. Some authors suggest possible difficulties in analyzing p53 protein in this type of biological material, mainly due to interfering effects of heterophilic antibodies [19]. This could potentially cause false positive or negative results. However, it has been suggested that the addition of mouse serum to analyzed samples helps to neutralize the possible effect of heterophilic antibodies [19]. When mouse serum is added to human plasma sample, heterophilic antibodies bind to proteins in mouse serum rather than to the primary antibody and thus decrease non-specific binding.

The aim of our study was to evaluate the effect of c-PAHs in ambient air on blood plasma levels of p53 and $p21^{WAF1}$ proteins in exposed and control subjects from three European cities—Prague, Kosice and Sofia. We also correlated the levels of both proteins with DNA adduct levels in lymphocytes of study subjects.

2. Material and methods

2.1. Chemicals

The primary anti-p53 antibody identifying both the mutated and the wild-type form of p53 (clone BP53-12) was purchased from Exbio (Czech Republic); the primary anti-$p21^{WAF1}$ antibody (clone HJ21) from Neomarkers; the secondary anti-p53 antibody (BMG-1B1) from Roche Diagnostics; the secondary anti-mouse IgG (NA931) from Amersham Pharmacia Biotech; p53 standard (sc-4246) and p21 standard (sc-4078 WB) from Santa Cruz Biotechnology Inc.; tetramethyl benzidine (TMB) substrate (T-8665) from Sigma; and immunoassay plates Immuno Module Maxisorp (no. 468 667) from Nunc.

2.2. Subjects and sampling

The study population consisted of 204 exposed subjects, policemen or bus drivers (males) working in the city centre and spending daily >8 h outdoors. The control subjects consisted of 152 healthy male volunteers spending >90% of daily time indoors and working in the suburban areas. Each participant completed a questionnaire on personal medical history and lifestyle. All participants were followed in winter due to high air pollution levels during this season.

All participants signed an informed consent form and could cancel their participation at any time during the study according to Helsinki II declaration. The study was approved by the ethical committee of the Institute of Experimental Medicine AS CR in Prague. 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 the working shift, by venipuncture into vacuettes containing sodium heparin, coded and transported to the laboratory in Prague, Kosice or Sofia. The samples were processed within 2 h and were kept in aliquots at −80 °C. For the analysis, the samples were shipped frozen to Institute of Experimental Medicine in Prague.

Subjects' exposure to c-PAHs was carried out by personal monitors used by the studied individuals during working shifts. Quantitative chemical analysis of c-PAHs, (benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenzo[*a*,*h*]anthracene, benzo[*g*,*h*,*i*]perylene and indeno[1,2,3-*cd*]pyrene) were determined by HPLC with fluorescence detection (for details on subjects and sampling see Ref. [5]).

2.3. ELISA assay

The levels of p53 and $p21^{WAF1}$ proteins in blood plasma were analyzed as described previously [15]. Briefly, for analysis of p53 protein, the plate was coated with 50 μl per well of