EXPERIMENTAL / LABORATORY STUDIES

Effect of Environmental Exposure to PAHs on Somatic Chromosomes

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Abstract: Polycyclic aromatic hydrocarbons (PAHs) are one of the main environmental pollutants in urban areas. Cytogenetic analysis of chromosomal aberrations is of great concern as they are involved in the mechanism of carcinogenesis. The aim of this study was to assess the genotoxic effect of occupational exposure to PAHs in a group of 30 Bulgarian traffic policemen compared to 30 office clerks.

Structural and numerical chromosome aberrations were analyzed by the conventional method in 100 to 300 cells per person. The exposure assessment was performed by personal sampling of air in the respiratory area. PAHs were identified by high performance liquid chromatography (HPLC) with a fluorescent detector.

SPSS 8.0 and Statistica 4.3 were used for statistical analysis of the results. The frequency of chromosomal aberrations in peripheral blood lymphocytes was higher in the occupationally exposed policemen - 2.55% than in the control group 1.57% (P < 0.002). The exposure to PAHs varied from 24.69 ng/m^3 to 203.97 ng/m^3 in the policemen and from 4.89 ng/m^3 to 120.61 ng/m^3 in the controls. PAHs possessed cytogenetic effects in highly exposed persons. The registered adverse effects increase the health risk for people professionally exposed to PAHs.

Key Words: chromosome aberrations, PAH-exposure, traffic policemen

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are significant environmental pollutants representing an important risk factor in human cancers. They are a large class of organic compounds including benzo [a] pyrene, benz.fluoranthene, naphthalene and lacetonaphthene, which are widely disseminated in the environment. The most important sources of PAHs in urban areas are traffic vehicle, tobacco smoking, food and wood processing and a number of production proceses associated with the partial burning out of organic substances.

PAHs are chemical carcinogens whose active metabolites form DNA adducts. They are potentially toxic, mutagenic and carcinogenic. The carcinogenic effect of toxic substances depends on their interaction with cellular constituents, including proteins, lipids and most important with nucleic acids, the genetic material of the cell. DNA is the ultimate molecule of life and with histones and nonhistones it builds the structure of the chromosomes.

A variety of different primary lesions are induced in cellular DNA by chemical mutagenes: single-strand and double-strand breaks, base damage of different types, DNA-DNA and DNA-proteins crosslinks, radical formations intercalations, formation of bulky adducts etc.

DNA damage can be repaired to give an apparently normal chromosome. Alternatively, it can be misrepaired to form an exchange or remain unrepaired in a terminal deletion.

It is generally accepted that chromosomal mutations are causal events in the development of neoplasia and it has been postulated that increased cytogenetic damage may be an indication of an enhanced cancer risk (1). Chromosomal damage plays an important role in the activation of proto-oncogenes, and their interactions play an indirect part in the formation of malignancy (2).

Chromosomal aberrations (CAs), sister chromatid exchanges (SCEs), and micronuclei (MN) in peripheral blood lymphocytes (PBLs) have been used as cytogenetic biomarkers for assessment of genotoxic risks in occupationally exposed people. The idea that increased cytogenetic damage indicates an enhanced cancer risk was the conceptual basis for this application. Nordic and Italian cohorts were established to evaluate this hypothesis (3). Previous analysis showed a positive trend between CAs frequency and increased cancer risk. Many investigators showed that the frequency of CAs in PBLs is a relevant biomarker for cancer risk in humans, reflecting either early biological effects of genotoxic carcinogens or individual cancer susceptibility (3). The hypothesis that the extent of genetic damage in PBLs is related to similar events in the precursor cells for carcinogenic processes in the target tissues was the conceptual basis for this biomarker.

There is heavy traffic in Sofia, which pollutes the air substantially. In recent years, the incidence of cancer has increased (4). Lung cancer, which is mostly related to air pollution and smoking, is more common in big cities. In urban areas, exposure to low levels and short-term peak levels of engine exhaust is ubiquitous. Higher exposure to engine exhausts may occur in occupations such as the traffic police.

The aim of this study was to assess the genotoxic effect of occupational exposure to PAHs in a group of traffic policemen in Sofia. CAs in PBLs were used as a biological marker for genotoxic effects.

The present study is a part of a more comprehensive investigation on the influence of PAHs on the health of professionally exposed people.

Materials and Methods

Subjects

Thirty subjects were selected from among town policemen usually on duty in busy streets in 8-10 h shifts. Their average age was 33 years. All of them were exposed to PAHs in the range of the exposure limit.

The control group consisted of 30 office clerks working indoor in different buildings without occupational exposure. Their average age was 36 years. In order to avoid sex differences all investigated subjects were male.

All subjects completed a questionnaire to assess general health, life style, years of employment, smoking and alcohol drinking habits, diets (smoked and grilled meat), and use of medication.

Ethics

The protocol and informed consent documents were reviewed and approved by the local ethics committee. The consent forms were prepared in conformance with the Declaration of Helsinki and local country laws. All participating volunteers were asked to provide written consent after receiving an explanation of the study.

Exposure assessment

An average shift sampling (long-term) was performed using charcoal sorbent tubes (type NIOSH) with low-flow personal sampling pumps, type Compur 4903, Germany, and Gilian 113 PS, USA, with debit of 20-30 cm^3/min. PAHs were identified by a gas chromatograph with a mass spectrometric detector. High performance liquid chromatography (HPLC) with a fluorescent detector and a Prosphere 300 PAH 5 column were used for quantitative determination. The detection limit of the method for benzo [a] pyrene is 0.1 ng/m^3.

There are peaks in the chromatograms of all 16 PAHs. The air pollution results are presented as tPAHs (total PAHs) and separately for the leading pollutant and proven carcinogen benzo [a] pyrene (BaP).

The investigation was performed in winter, during the highest exposure to PAHs.

Cytogenetic endpoint

The conventional method was used for the detection of structural and numerical chromosomal aberrations in the PBLs of the subjects (5). It is used as a biological group test for an evaluation of the exposure of the subjects to genotoxic substances with clastogenic effects. Venous blood was collected in vacutainers containing Li-herapin and lymphocyte cultures were incubated at 37 °C in 4.25 ml.RPMI + 0.75 ml of fetal bovine serum and

phytochaemaglutinin P. Colcemid was added to the cultures at 46 h after incubation. The cells were harvested at 48 h. After hypotonic treatment with KCL (0.075 M) the cells were fixed with methanol–acetic acid (3:1) and spread on clean slides. From 100 to 300 cells were scored per person for structural and numerical chromosomal aberrations after staining with 5% Giemsa. Parts of the slides were frozen at –20 °C for FISH staining.

Statistical analysis

SPSS 8.0 and Statistica 4.3 were used for statistical analysis of the results. One-way ANOVA was used to compare the means among groups with normal distribution. In groups with abnormal distribution the results were assessed using the Mann-Whitney test. The correlation analysis was estimated according to Pearson and Spearman.

Results

Sixty subjects were sampled for tPAHs. The exposure of policemen varied from 24.69 ng/m^3 to 203.97 ng/m^3 and of the controls from 4.89 ng/m^3 to 120.61 ng/m^3. Exposure to the most important pollutant BaP for the policemen varied from 0.14 ng/m^3 to 8.42 ng/m^3 and for the controls from 0.11 ng/m^3 to 5,08 ng/m^3.

The mean values of the tPAHs were 36.86 ± 5.69 ng/m^3 and of the BaP were 1.82 ± 0.21 ng/m^3 for the controls, respectively, and 73.02 ± 7.90 ng/m^3 and 3.66 ± 0.40 ng/m^3 for the policemen (Table). The comparison between the mean concentrations of tPAHs and BaP in the 2 groups is shown in Figure 1.
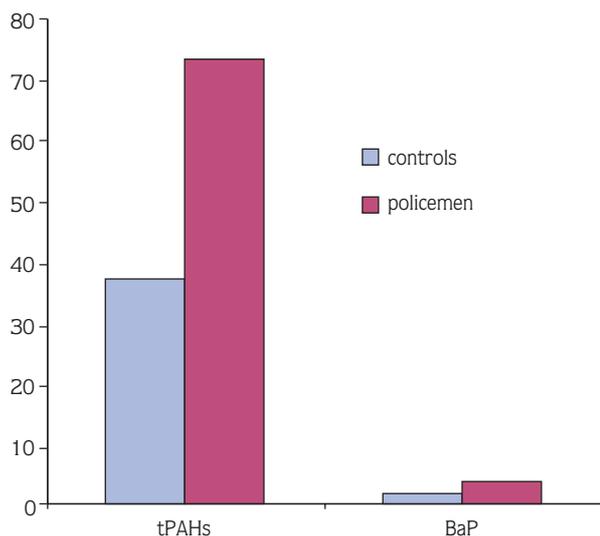

Figure 1. Comparison between the mean concentrations of PAHs and BaP in the 2 groups.
Legend:
tPAH- total concentration of PAHs
BaP- concentration of benzo [a] pyrene

The frequency of CAs (excluding gaps) in the exposed group varied from 1% to 6% in different subjects and from 0% to 3% among the control subjects. The mean value of CAs for the office clerks was 1.57 ± 0.77 and for the exposed group 2.55 ± 1.33 (Table).

Statistical analysis (excluding gaps), indicated that differences in the frequencies of chromosomal damage between the exposed and nonexposed group were highly significant (P = 0.002).

In total 4700 metaphases were scored in the control group and 37 chromatid breaks and 41 chromosome breaks were found.

Table. Mean value of the concentration of tPAHs, BaP and the frequency of chromosome aberrations in both groups.

Groups	Exposure		Chromosome aberrations	
	tPAHs	BaP	Ab.C/%	B.C./%
	X ± SD	X ± SD	X ± SD	X ± SD
Controls	36.86 ± 5.69	1.82 ± 0.21	1.57 ± 0.77	0.2 ± 0.1
Traffic policemen	73.02 ± 7.90*	3.66 ± 0.40**	2.55 ± 1.33***	0.3 ± 0.2****

*P < 0.0001   **P = 0.001   ***P = 0.002   ****P = 0.003
Legend:
Ab. C.- aberrant cells
B. C.- breaks per cell

In the group of policemen 5700 cells were analyzed. Total CAs included 65 chromatid breaks, 68 chromosome breaks, 3 chromatid exchanges and 4 dicentric chromosomes. Dicentric chromosomes were present in only 2 subjects. Ring chromosomes were not found in either groups. The comparison between frequencies of CAs in the 2 groups is shown in Figure 2.
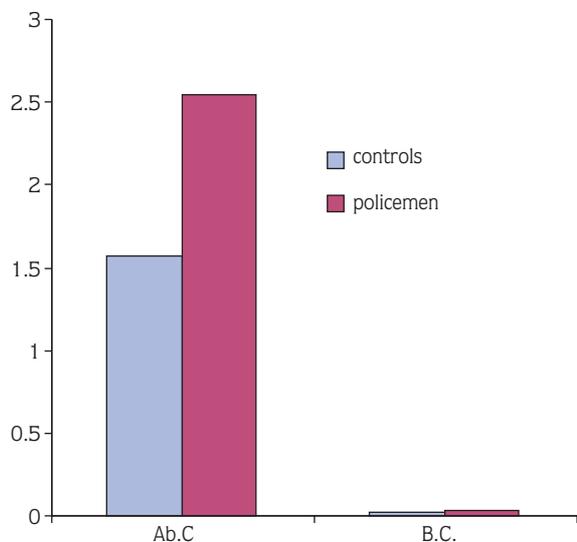

Figure 2. Comparison between frequency of chromosome aberrations in the 2 groups.
Legend:
Ab.C.- aberrant cells
B.C.- breaks per cell

Statistical analysis shows a correlation between the frequency of CAs and the concentration of total PAHs and BaP. The correlation was highly significant ($P < 0.0001$). The lineal regression model shows that an increase in BaP concentration leads to an increase in the frequency of chromosome aberrations (Figure 3):

$$Y = 0.877 + 0.431 \cdot X, \ P < 0.0001$$

The lineal dependency for PAH concentration is presented in Figure 4:

$$Y = 1.137 + 0.0168 \cdot X, \ P < 0.0001$$

Discussion

Our study indicated that there is a significant increase in the frequency of CAs in the PBLs of policemen who have been occupationally exposed long-term to PAHs. The most commonly detected abnormalities were gaps and
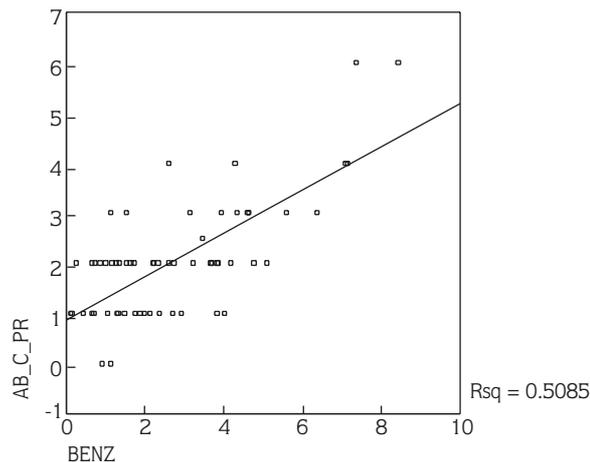

Figure 3. Correlation between total chromosomal aberrations (%) and concentration of BaP.
Legend:
AB_C_PR- aberrant cells,%
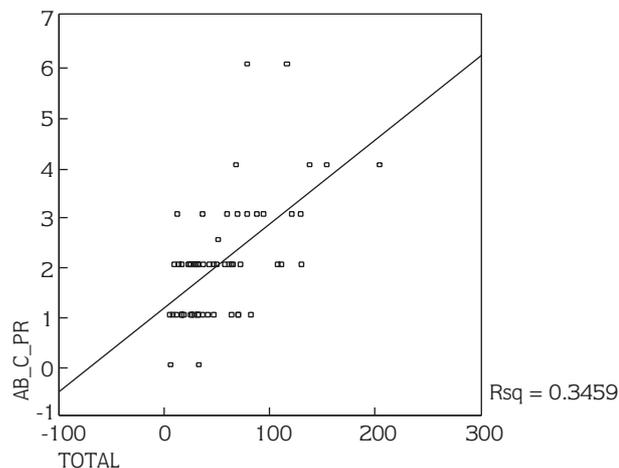BENZ- concentration of BaP
$Y = 0.877 + 0.431 \cdot X$

Figure 4. Correlation between total chromosomal aberrations (%) and concentration of total PAHs.
Legend:
AB_C_PR- aberrant cells (%)
TOTAL- total PAHs
$Y = 1.137 + 0.0168 \cdot X$

breaks. Rearrangements, as chromosome and chromatid exchanges, were also increased in the policemen. The elevated chromosome damage in the investigated group compared to the controls can be explained by their occupational exposure to pollutants from automobile exhaust. The main relevance of lymphocyte chromosome damage is its indicative value for genotoxic exposure, which is considered to be related with the multistage process of carcinogenesis (6).

PAHs are metabolized by cytochrome P450-dependent monooxygenase enzymes to a wide variety of primary metabolites, including epoxides, dihydrodiols, quinones, and phenols (7-10). A number of the products of initial oxidation (i.e. phenols and diols) can be reoxidized and recycled through these same metabolic pathways (7-10). Of particular interest is the formation of the highly reactive PAH diol-epoxides. Studies in a variety of tissues and species of the ubiquitous PAH, BaP, for example, have shown that it is activated to mutagenic and carcinogenic derivatives by a 2-step mechanism leading to the formation of a "bay-region" diol-epoxide (9,10) and that the various isomers of this diol-epoxide bind covalently to different extents at various positions on DNA bases, particularly on deoxyguanosine (dGuo) residues (9-11). This 2-step oxidation mechanism appears to be a major pathway of metabolic activation of BaP and other PAHs for tumor initiation in mouse skin (11,12).

The majority of studies investigating the influence of air pollution on human chromosomes showed positive results. An increased frequency of CAs was found in the PBLs of workers in the rubber industry, taxi drivers, coal tar and coke oven workers, etc. (13-20). Our results are in accordance with these findings.

A significant correlation was discovered between the frequencies of CAs and concentration of BaP and PAHs. Interestingly, we found a significant correlation between the increase in CAs and tobacco smoking. Kasuba et al. studied the cytogenetic effects of air pollutants in PBLs in occupationally exposed people and found no significant difference in chromosome damage between smokers and nonsmokers (15), while Anwar reported an influence of smoking habits on CA increases (13). The high incidence of chromosomal abnormalities in our study is mostly related to high concentrations of PAHs in the working environment.

Numerous investigators have shown that there is a significant correlation between malignancies and chromosomal damage (2,21-23). Sorsa et al. reported that increased frequencies of CAs can serve as an indicator of exposure to genotoxic agents and as a signal for the potential of cancer risk at the group level (24). Hagmar and Bonassi have shown that an elevated CA frequency in PBLs from healthy subjects is related to the increased frequency of malignant disease, and it has a predictive value for increased cancer risk (3).

In conclusion, there is a significant increase in the frequency of chromosome aberrations in peripheral blood lymphocytes from professionally exposed traffic policemen compared to the control subjects.

PAHs possessed cytogenetic effects in the PBLs of occupationally exposed policemen. The registered adverse cytogenetic effect increases the health risk for people professionally exposed to PAHs, indicating that preventive health measures must be taken.

Vegetable consumption may play a protective role by influencing the mechanism of metabolic activation of carcinogens. It is known that cruciferous vegetables contain indoles and isothiocyanates, which affect the metabolism of PAHs (25). This could lead to inhibition of the carcinogenic potential of the chemicals and risk reduction. Soy sauce and other antioxidant substances may be related to an anticarcinogenic effect as well (26).

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