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Exposure to environmental polycyclic aromatic hydrocarbons: Influences on cellular susceptibility to DNA damage (sampling Košice and Sofia)

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Abstract

The aim of this study was to investigate a possible influence of occupational exposure to carcinogenic environmental polycyclic aromatic hydrocarbons (c-PAHs) on cellular susceptibility to the induction of the DNA damage. Monitoring was performed and blood samples were collected from two groups of male subjects: occupationally exposed and matched controls. The group exposed to c-PAHs (average age of 35.1 years) consisted of 52 policemen from Košice and 26 policemen and 25 bus drivers (51 altogether) from Sofia. The control group (average age of 36.4 years) consisted of 54 unexposed subjects from Košice and 24 from Sofia. In the investigated groups 52.5% of exposed subjects and 45.3% of control were current smokers. A challenging dose of X-rays (3 Gy) and an alkaline version of the single cell gel electrophoresis (SCGE) assay, known as Comet assay, were used to evaluate levels of induced DNA damage and repair kinetics in isolated human blood lymphocytes. DNA damage detected in lymphocytes prior to or after irradiation did not differ significantly between exposed and unexposed subjects. A significant decrease in repair efficiency due to exposure to PAHs was observed in the exposed individuals from Košice and Sofia, when analysed separately or together. A negative influence of tobacco smoking on the efficiency of DNA repair was observed. Statistically significant differences were found between subgroups stratified according to education level in Sofia: the half times for DNA repair declined with the increasing level of education. These results confirm that environmental exposure to c-PAHs can alter the ability of blood lymphocytes to repair DNA damage and, as a result could potentially lead to effects that are hazardous to human health.

Keywords: PAHs; DNA repair; Kinetics; SCGE assay; Polymorphism; Life style factors

Abbreviations: c-PAHs, carcinogenic polycyclic aromatic hydrocarbons; B[*a*]P, benzo[*a*]pyrene; SCGE, single cell gel electrophoresis assay; T-DNA, tail DNA, percentage of the DNA in the comet tail; TM, tail moment, percentage of DNA in the tail multiplied by the tail length; $RD_{T\text{-}DNA}$ or RD_{TM}, residual (unrepaired) damage as a percentage of initial DNA damage estimated from T-DNA or TM parameters; $T_{1/2}$, half time of the DNA repair process

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1. Introduction

All living organisms are unavoidably exposed to various environmental genotoxins and changes in the genetic material may be expected as the results of this exposure [1]. Studies have shown that several polycyclic aromatic hydrocarbons (PAHs) are human carcinogens [2,3]. The aim of EC project EXPAH was to investigate effects of carcinogenic PAHs (c-PAHs) present in environmental pollution on endogenous and exogenous DNA damage. The aim of this study, a part of the EXPAH project, was to investigate a possible influence of exposure to environmental PAHs on cellular susceptibility to the induction of oxidative types of DNA damage [4–6].

Human biomonitoring as a tool to identify health risk originating from environmental exposures has gained increasing interest in many fields associated with health risk assessment. A biomarker based on a fast, sensitive and reliable method for the detection of environmentally induced DNA damage and/or of the ability of cells to repair DNA damage might be crucial to many health-related fields, from molecular epidemiology to preventive and clinical medicine. In this study, we proposed a DNA repair competency assay to study variation amongst subjects in their cellular response to a challenging dose of X-rays and in the subsequent ability of cells to repair the induced DNA damage [6]. Ionizing radiation was used as the agent inducing, amongst other types of DNA damage, oxidative types. An alkaline version of the single cell gel electrophoresis (SCGE) assay was used to analyse levels of DNA damage and its repair. Evidence exists that levels of DNA damage detected by the SCGE assay, also known as the Comet assay, correlate on the one hand with the physico-chemical measures of the genotoxins concentrations, and on the other hand, with the chromosome aberrations expressed during the following mitosis [4]. Those are well-known biomarkers associated with adverse health outcomes and with cancer in particular [7,8]. Studies were carried out on blood lymphocytes sampled from a population occupationally exposed to environmental PAHs and from matched controls, all male volunteers from two cities: Košice in the Slovak Republic and Sofia in Bulgaria.

2. Material and methods

2.1. Sampling

All subjects were volunteers, and were young males who reported themselves as generally healthy men, with no apparent symptoms of any disease. As reported elsewhere [5], during the work shift, personal monitoring of exposures was conducted by the use of personal monitors, which collected respirable particulate matter PM2.5 (of aerodynamic diameter $< 2.5\,\mu m$). The c-PAHs were extracted from filters and analysed for the presence of benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene (B[*a*]P), dibenzo[*a*,*h*]anthracene, benzo[*g*,*h*,*i*] perylene and indeno[1,2,3-*cd*]pyrene by the high performance liquid chromatography (HPLC) with fluorescence detection. Information describing each subjects' health and social status, education, main habits and life styles was collected through questionnaires [5].

The exposed group consisted of 52 males from Kosice, all of whom were policemen, and 51 males from Sofia, 26 of which were policemen and 25 of them were bus drivers. The average age of this group was 35.1 years. The group of matched controls, with an average age 36.4 years, consisted of 54 males from Kosice and 24 males from Sofia. Amongst these groups 52.5% of the exposed and 45.3% of the control subjects were current smokers. For each subject, lymphocytes were isolated from fresh whole blood samples, which were then frozen and transported (on dry ice) to the laboratory of the Department of Radiation and Environmental Biology (DREB), Institute of Nuclear Physics PAN in Kraków, Poland, where aliquots were stored at $-80\,^{\circ}C$ until used to evaluate for DNA damage.

2.2. Repair competence assay with X-rays as a challenging treatment

Vials with frozen cells were submerged, according to the procedures described elsewhere [9,10], in a $37\,^{\circ}C$ water bath, until the last trace of ice was melted. Then, cells were quickly transferred to centrifuge tubes containing 15 ml of a thawing medium (50% fetal calf serum, 40% RPMI 1640, 10% Dextrose), centrifuged for 10 min at $200 \times g$, re-suspended in 3 ml of cold RPMI 1640. At this stage, the viability of the cells was determined using trypan blue technique. The lymphocytes were than divided into the following groups:

- Analysis of background levels of DNA damage in lymphocytes, presumed to represent the DNA damage induced *in vivo*.
- Analysis of levels of DNA damage present in lymphocytes immediately after irradiation with a challenging dose of X-rays, considered to reflect cellular sensitivity.
- Analysis of levels of unrepaired DNA damage present in lymphocytes after an incubation period sufficient to allow the irradiated cells to complete the fast component of repair process, presumed to reflect the rate of DNA repair process.

In the subgroup of subjects from Sofia, apart from the cellular sensitivity were investigated the efficiency of the DNA repair process and its kinetics. For that purpose, each aliquot of irradiated lymphocytes was divided into several parts and each part was cultured for different periods of time. Fig. 1 shows examples of typical kinetics observed in the majority