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Influence of environmental exposure to PAHs on the susceptibility of lymphocytes to DNA-damage induction and on their repair capacity

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Abstract

The influence of occupational exposure to environmental carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) on DNA damage detected in lymphocytes of exposed people (city policemen) was studied. The cellular susceptibility to the induction of the DNA damage and the repair capacity of exposed donors are presented in comparison with matched controls. Monitoring was performed and blood samples (164 donors) were collected in Prague, Czech Republic, during the winter and summer seasons. The single-cell gel electrophoresis (SCGE) assay with an internal standard was applied to evaluate the DNA damage. A challenging dose of 2 Gy of X-rays was used to study cellular capacities. In the results of studies of the DNA damage induced in vivo or as an immediate response to the challenging treatment no significant difference was found between exposed and unexposed subgroups. The percentage of non-repaired X-ray-induced DNA damage (residual damage, RD) overall in both seasons was significantly higher in lymphocytes of policemen exposed to c-PAHs than in matched controls ($RD_{T\text{-}DNA}$, %DNA in the comet tail: winter 36.4 ± 22.1 versus 22.7 ± 10.8, $p < 0.001$; summer 47.7 ± 22.9 versus 34.7 ± 15.2, $p < 0.001$). The results suggest that occupational exposure to environmental c-PAHs significantly reduces the cellular capacity to repair the DNA damage induced by a challenging treatment. A significant decrease of repair efficiency in donors occupationally exposed to environmental c-PAHs was also observed when subgroups were stratified according to smoking history. In conclusion, our results suggest that environmental exposure to c-PAHs affects the cellular repair processes and can lead to harmful effects hazardous to human health.

Keywords: DNA repair; Carcinogenic PAHs; Single-cell gel electrophoresis assay; Policemen

Abbreviations: B[*a*]A, benz[*a*]anthracene; B[*a*]P, benzo[a]pyrene; B[*b*]F, benzo[*b*]fluoranthene; B[*g*,*h*,*l*]P, benzo[*g*,*h*,*l*]perylene; B[*k*]F, benzo[*k*]fluoranthene; CHRY, chrysene; c-PAHs carcinogenic polycyclic aromatic hydrocarbons; DB[*a*,*h*]A, dibenzo[*a*,*h*]anthracene; I[*c*,*d*]P, indeno[1,2,3-*cd*]pyrene; IS, internal standard, sampling probe collected from a healthy male donor, "Mister Standard"; LMA, low melting agar; NMA, normal melting agar; $RD_{T\text{-}DNA}$ or RD_{TM}, a percentage of residual damage estimated from T-DNA or TM parameters; SCGE, single-cell gel electrophoresis assay; SF, standardizing factor; 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; TL, a length of the comet tail measured from the edge of the comet head

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are considered as potential human carcinogens, which is why the effects of exposure to carcinogenic PAHs (c-PAHs) present in environment are investigated in many studies [1–3]. Dozens of studies have led to a general confidence that c-PAHs may trigger mutagenesis and subsequent pathological processes, including carcinogenesis [4,5]. Great progress has also been made in understanding the effects of many environmental mutagens and carcinogens on important cellular functions, such as the transcriptional induction and phosphorylation of many proteins involved in DNA repair, signal transduction, cell cycling and apoptosis [6]. Although some of the cellular responses are transient, it is likely that they have evolved to deal with the potentially deleterious effects produced by DNA damage. Mutations producing a selectable phenotype in surviving cells may become apparent only many cell-generations later. It is generally known that various mutagens and carcinogens induce a unique mutation spectrum. However, there are many factors that may affect the mutational outcomes of DNA-damaging agents. Moreover, the sequence selectivity of the DNA damage and the repair processes can also contribute greatly in shaping the mutation spectrum of a DNA-damaging agent [6]. Therefore, studying DNA damage and its repair remains an important key-factor in understanding the biological effects of mutagens and carcinogens.

The aim of our study was to investigate whether occupational exposure to c-PAHs may affect cellular susceptibility to the induction of the DNA damage and the capacity to repair it.

We have applied the alkaline version of the single-cell gel electrophoresis (SCGE) assay to evaluate the DNA damage induced in both conditions; in vivo (detected in lymphocytes isolated from whole blood samples) and in studies in vitro [7,8]. The SCGE assay, also known as the Comet assay, has been widely used to detect DNA damage in cells exposed to various physical or chemical agents. At high pH levels this method permits the detection of a wide spectrum of DNA damage including single- and double-strand breaks, or alkali-labile sites [9,10]. In molecular epidemiology studies, the DNA damage evaluated by the Comet assay is considered as a non-specific biomarker of genotoxic exposure [9].

A challenging dose of X-rays and a repair-competence assay were proposed to investigate the inter-individual variation in the response to damage induced by agents present in the environment. Ionizing radiation is known as an environmental agent inducing among others free radicals and oxidative types of damage [8]. Radiation is also responsible for single- and double-strand breaks, or alkali-labile sites in DNA, which are easily detected by the SCGE assay. Evaluation of the repair efficacy was done by comparing the DNA damage detected immediately after the challenging exposure with the residual damage detected in irradiated cells after the incubation allowing the cells to complete the repair process. The half-life of the repair process in lymphocytes of young healthy male donors estimated from kinetic studies is ∼5 min [8]. Therefore, incubation of irradiated lymphocytes for a period longer than 1 h should not decrease anymore the amount of residual damage detected by the SCGE assay. The distribution in the investigated groups of the damage detected in response to challenging dose and the residual damage (non-repaired damage) expressed as the percentage of the induced initial damage should express firstly the variation between individuals in vulnerability of their DNA to the induction of damage and, secondly, the efficiency of the repair process [8].

2. Material and methods

2.1. Subjects

All donors in this study were young males living in the city of Prague, Czech Republic, who were reporting themselves as generally healthy, with no apparent symptoms of any disease. Samplings of donors were done in two seasons (winter and summer). The investigated group consisted of donors occupationally exposed to the environmental c-PAHs (working as city policemen; average age, 32.1 years). Persons from the control group were matched for age (on average 29.4 years in both seasons' samplings), unexposed occupationally to environmental c-PAHs. Information about donors' health and social status, education, main habits and lifestyles were collected through questionnaires.

A brief description of the study groups (samples of lymphocytes from 164 donors) is presented in Tables 1 and 2. Ambient-air and personal monitoring of exposure were performed under the EC EXPAH project and reported elsewhere [11,12]. Mean values of the concentration of benzo[*a*]pyrene and the sum of eight carcinogenic PAHs (benz[*a*]anthracene (B[*a*]A), chrysene (CHRY), benzo[*b*]fluoranthene (B[*b*]F), benzo[*k*]fluoranthene (B[*k*]F), benzo[*a*]pyrene (B[*a*]P), dibenzo[*a,h*]anthracene (DB[*a,h*]A), benzo[*g,h,l*]perylene (B[*g,h,l*]P) and indeno[1,2,3-*cd*]pyrene (I[*c,d*]P)) detected in the collected air samples are presented in Tables 1 and 2. Table 1 presents the description of both groups investigated in two seasons stratified according to the occupation of the donors. In Table 2 those groups are stratified according to smoking habits and occupation.