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Repair competence assay in studies of the influence of environmental exposure to c-PAHs on individual susceptibility to induction of DNA damage

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Abstract

Previous results from studies performed in three European cities suggested a decrease in DNA repair efficiency observed in lymphocytes of subjects occupationally exposed to environmental carcinogenic polycyclic aromatic hydrocarbons (c-PAHs). The aim of this study was to investigate whether a relationship between exposure to environmental c-PAHs and cellular vulnerability to the induction of DNA damage and its repair is confirmed in a pooled group of subjects from Prague, Košice and Sofia. The investigated pool consisted of 144 subjects occupationally exposed to environmental c-PAHs, who were municipal policemen or bus drivers. A control group of 115 matched individuals consisted of males unexposed at work to c-PAHs. The repair efficacy was evaluated by a comparison of the DNA damage detected by the single cell gel electrophoresis (SCGE) immediately after challenging the cells with X-ray irradiation, with residual damage (RD) being measured after an incubation period of 60 min. A stochastic concept for a mechanism of the interaction between DNA and various genotoxic exposures, was applied to analyze a relationship between exposure and biological effect in the studied sample. The outcome of the study confirms that the exposure to environmental c-PAHs or smoking cigarettes, significantly decreases DNA repair efficiency (repair efficiency in the pooled group of exposed individuals was $61.8 \pm 11.8\%$ versus 67.9 ± 9.9 in control, $p < 0.001$, and repair efficiency in group of smoking individuals was $63.0 \pm 11.5\%$ versus 65.9 ± 11.1 in nonsmokers, $p < 0.005$). The repair efficiency can be affected by a genetic polymorphism, such as subjects with a homozygous mutation in polymorphic CYP1A1$_{(Val/Val)}$ enzyme, or slow NAT2 acetylators, who showed a considerably lower

Abbreviations: c-PAHs, carcinogenic polycyclic aromatic hydrocarbons; B[*a*]A, benzo[*a*]anthracene; CHRY, chrysene; B[*b*]F, benzo[*b*]fluoranthene; B[*k*]F, benzo[*k*]fluoranthene; B[*a*]P, benzo[*a*]pyrene; DB[*a,h*]A, dibenzo[*a,h*]anthracene; B[*g,h,l*]P, benzo[*g,h,l*]perylene; I[*c,d*]P, indeno[1,2,3-*cd*]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; $RE_{T\text{-}DNA}$ or RE_{TM}, repair efficiency as a percentage of repaired DNA damage estimated from T-DNA or TM parameters; CEF, combined exposure factor ($CEF_{B[a]P,cot}$—based on results from B[*a*]P and cotinine monitoring; $CEF_{c\text{-}PAH,cot}$, —based on results from c-PAHs and cotinine monitoring); IS, internal standard; T-DNA_0, T-$DNA_{X\text{-}rays}$, DNA damage estimate based on the measurements of the percentage of the DNA in the comet tail in cells with no (0) treatment in vitro or in a response to challenging treatment with X-rays respectively); SH, smoking habit

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DNA repair efficiency (i.e. average repair efficiency in subgroups of fast acetylators was for the control subgroup 68.1% versus 66.5% in exposed subjects, while in the case of subgroups of slow acetylators, for the control group was 68.0% versus significantly less in the exposed subjects, 60.6%, $p < 0.05$). Smoking habits, or the diet's vitamin content, significantly affected the process. The results obtained confirm a potential value of the method as a biomarker of susceptibility in molecular epidemiology or preclinical studies, aimed at predicting susceptibility to various genotoxic exposures (environmental, occupational, therapeutic). To conclude, the research proved the influence of environmental c-PAHs, genotypes, and life styles on DNA damage and on its repair efficiency. Even low exposure to environmental c-PAHs altered DNA repair abilities of the subjects, which may result in an increased cancer risk. The findings confirm that c-PAHs should become pollutants that are subject to regulation.

Keywords: DNA repair; Impact of genetic polymorphisms; Carcinogenic c-PAHs; Single cell gel electrophoresis assay; Policemen; Stochastic approach

1. Introduction

All types of genomic injury induced by environmental carcinogens are possible triggers of mutagenesis and carcinogenesis. Humans and living organisms are unavoidably exposed to various environmental genotoxins. Despite intensive research on airborne organic particulates, especially their content of polycyclic aromatic hydrocarbons (PAHs), it is estimated that about 90% of the atmospheric organic pollutants had never been determined [1]. The c-PAHs are metabolized to a mixture of epoxides, involving the biological activation of the parent compound to produce the diol-epoxide metabolite, a potent mutagen and carcinogen. c-PAHs (e.g. B[*a*]A—benzo[*a*]anthracene, CHRY—chrysene, B[*b*]F—benzo[*b*]fluoranthene, B[*k*]F—benzo[*k*]fluoranthene, B[*a*]P—benzo[*a*]pyrene, DB[*a*,*h*]A—dibenzo[*a*,*h*] anthracene, B[*g*,*h*,*l*]P—benzo[*g*,*h*,*l*]perylene, I[*c*,*d*]P—indeno[1,2,3-*cd*]pyrene) occur as environmental agents, and are known to attack DNA, and to induce primary or secondary DNA damage, even single or double strand lesions or breaks. Another important aspect is that primary sequence, chromatin structure, methylation, protein association, and transcriptional activity can affect an initial level and distribution of DNA damage, as well as its repair efficiency. Smoking is also known as a factor having an impact on c-PAHs exposure, and is known to be responsible for an induction of DNA adducts. Therefore, individuals differ in terms of their DNA's vulnerability and repair efficiency and capacity [2].

Various biomarkers can be used to follow the events to elucidate mechanisms of the genotoxic/carcinogenic process, as well as the individual response to carcinogens. It had been already suggested that the changes in the blood cells' repair capacity could be a better biomarker for chronic low-dose exposure to ionizing radiation and a better indicator of individual sensitivity to mutagens, than an induced DNA damage determination [3].

During the studies performed under the EC EXPAH project, groups of subjects occupationally exposed to environmental c-PAHs and matched controls were investigated. Results of monitoring performed for evaluation of exposure in those studies, personal monitoring and genotyping have been reported already elsewhere [4–6]. The DNA repair competence was investigated in the lymphocytes of studied subjects by challenging cells with a radiation dose. Ionizing radiation was applied as an environmental agent known to induce free radicals and a wide spectrum of DNA damage, including single and double strand breaks, base damage, and oxidative types of damage [7]. The whole blood samples were collected at various sites and lymphocytes were isolated and frozen, transported to Poland, to the laboratory of the Department of Radiation and Environmental Biology at the Institute of Nuclear Physics in Krakow, where cells were stored at −80 °C before studies in vitro. For repair competence studies, lymphocytes were defrosted according to the standard procedure and viabilities of cells were evaluated. An alkaline version of the SCGE assay (also known as Comet assay) was used to detect DNA damage in the investigated cells [5,7].

Independent reports from the groups examined under the EXPAH project in Prague, Czech Republic, Košice, Slovak Republic and Sofia, Bulgaria revealed significant variations of individuals' DNA vulnerability based on the measurements of direct DNA damage and the residual damage [5,6]. Studies showed environmental exposure to c-PAHs, or smoking, may significantly reduce subjects' cellular DNA repair capacities.

The aim of this study was to investigate in the pooled sample from all sites, a relationship between exposure to environmental c-PAHs and cellular vulnerability to the induction and repair of DNA damage. According to the molecular theory of radiation biology, two independent