Influence of Occupational Exposure to PAHs on the Induction and Repair of DNA Damage Evaluated by the Alkaline Version of the SCGE Assay

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Single cell gel electrophoresis (SCGE) has been widely used to detect DNA damage of cells exposed in vitro and in vivo to various physical or chemical agents. In molecular epidemiology studies DNA damage evaluated by the comet assay is considered as non-specific biomarker of exposure effects [1]. However, attention has been paid to the experimental variability of this assay [2]. When large numbers of samples need to be analyzed, an internal standard is indispensable.

We have applied this method for studies of an influence of occupational exposure to polycyclic aromatic hydrocarbons (PAH) on the DNA damage detected in lymphocytes of exposed people, and cellular susceptibility to the induction of the oxidative damage. For the latter purpose the SCGE assay was applied to evaluate the DNA damage induced by 2 Gy of the challenging dose of X-rays (as an oxygen radicals and oxidative damage inducing agent), and again after a certain time of incubation during which a “completed” repair of the induced damage should significantly diminish the damage detected in the cells. For the purpose of proper adjustment of the residual (unrepaired) damage studies of the DNA repair kinetics were performed. The half life time of the repair process for a young male donor estimated from the kinetics was ~5 min, so, incubation of irradiated lymphocytes for the period longer than one hour did not decrease anymore the amount of residual damage.

Lymphocytes of the unexposed and exposed donors were divided into three parts for the estimate of the damage: a) induced in vivo, b) by challenging dose of X-rays, and c) a residual damage after repair during the incubation. To control stability of the assay experimental conditions, a group of cells from the same sampling probe of Mr Standard’s was divided into two parts for an analysis after challenging X-rays exposure and after repair of X-rays induced damage. To evaluate the DNA damage three parameters have been chosen (TL – length of the comet tail, tDNA – fraction of the DNA in the comet tail, TM – comet tail moment equal to percentage of DNA in the tail multiplied by the tail length). Experiments were run for the group of 100 donors from a reference group and persons exposed to PAHs (town policemen) from the Czech Republic.

Comparison of preliminary results showed a high reproducibility between an independent electrophoresis for the Mr Standard samples, and no significant difference between exposed and unexposed subgroups for various measures of the DNA damage induced in vivo. Preliminary results also suggest a difference between unexposed and exposed donors’ responses to radiation and the efficiency of the X-rays induced damage repair.

References:

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