

## Application of ionizing radiation in studies of biomarkers of individual susceptibility

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**Abstract** Human biomonitoring, as a tool to identify health risk from environmental exposures, has gained increasing interest especially in the areas of cancer risk assessment and diseases treatment. Chromosome aberrations resulting from direct DNA breakage or from inhibition of DNA repair or synthesis, measured in peripheral blood lymphocytes, have been used successfully in the assessment of health risk associated to environmental genotoxic exposures. A faster but sensitive and reliable method for detection of DNA damage, or DNA repair capacity, might be crucial to many fields from molecular epidemiology and toxicology to preventive and clinical medicine. There are reports that results of DNA measures with the use of single cell gel electrophoresis (SCGE) correlate, on the one hand, with physical measures of genotoxins, and on the other hand, with cytogenetic damage that is a biomarker associated to the alteration of the health risk. This review is based on studies in which exposure to radiation was applied as a challenging treatment and DNA damage induced and repaired was analyzed with the use of the alkaline version of SCGE assay. Results from studies on susceptibilities and repair competence carried out in various groups of exposed workers, controls, and cancer patients (more than 700 donors) show variability between donors both in a response to challenging treatment and in the efficiency of repair process. Influences of the occupational exposures and factors depending on genotypes or life style on cellular capacities are observed. Discussed results suggest that study *in vitro* with the challenging cells by radiation exposure and measuring, with the SCGE assay, the DNA damage before and after repair, may develop a good biomarker of the individual susceptibility to various genotoxins and exposures (environmental, occupational, therapeutic). Such a biomarker may have a potential use in a molecular epidemiology and preclinical identification.

**Key words** biomarkers • susceptibility • application of radiation • DNA repair

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### Introduction

Humans and living organisms are unavoidably exposed to various environmental genotoxins. Genotoxic exposures may result from endogenous mutagens, (via cellular metabolic processes associated with oxygen free radical induction) or from exogenous mutagens present in the environment (as a pollution of air, water and soil, e.g. motor-vehicle emissions, pesticides, industrial effluents), or in occupational exposures (e.g. pesticides, petrochemicals), or from diet (e.g. substances naturally present in the food, generated during cooking, or added for food preservation), life style (e.g. tobacco smoking, cosmetics), radiation (e.g. radon, diagnostic X-rays examinations) and medical procedures (e.g. chemotherapy, radiotherapy). The long list of genotoxins is consistent with evidence indicating that the great majority of cancer, in principle, is preventable because the factors that determine cancer incidence are largely exogenous or “environmental”. It has been estimated that perhaps 75–80% of all cancers in the United States

is due, at least partly, to environmental factors and, therefore, is potentially avoidable [17]. Human biomonitoring is a tool to identify and to quantify the potential risk of environmental exposures. In environmental epidemiology the biomarkers of effects are believed to represent events in a causal pathway to disease, their occurrence may be viewed as indicative of having significantly increased risk for disease [3, 16]. However, various individuals may deviate in both, in the vulnerability of their DNA to the induction of damage and in the efficiency to repair it [4]. To study the individual susceptibility to genotoxic agents present in the environment, a procedure of challenging cells by exposure to radiation and studying the repair competence was proposed [6]. Ionizing radiation is an environmental agent that is known as inducing, among others, the DNA strand breaks, free radicals and oxidative types of damage [13].

The presented review is based on studies in which cells were challenged by exposure to radiation and the DNA damage induction and repair were analyzed with the use of the alkaline version of SCGE assay. Cellular susceptibilities to the induction of the DNA damage and repair competence were studied in lymphocytes collected from various groups of exposed workers, controls, and cancer patients (more than 700 donors) [6–8, 10, 12]. Evaluation of the repair efficacy was done by comparing the DNA damage detected immediately after the challenge of cells, with the amount of the DNA damage detected as a residual damage, that was not repaired during the post exposure incubation.

The SCGE, also known as the Comet assay, has been widely used to detect DNA damage in cells exposed to various physical or chemical agents [1]. SCGE assay permits at high pH level to detect a wide spectrum of DNA damage including single and double-strand breaks, or alkali labile sites. The assay is often applied to various types of cells and there are findings reported that DNA damage detected in the investigated cells is highly correlated to the amount of chromosomal damage expressed in those cells during a consequent mitotic division [9]. Chromosome aberrations are known biomarkers of biological effects associated to cancer risk [5, 11].

The aim of this presentation is to consider whether results from the reported studies approve that a challenging of cells by irradiation, followed by the evaluation of cellular repair competence could be used to study variation between individuals in a cellular response to the environmental or therapeutic hazards. That in a consequence might be considered as a biomarker of individual susceptibility and used as a predictive assay for molecular epidemiology. Occurrence of the disparity in the DNA damage detected in cells responding to the challenging treatments, with the variation in residual DNA damage (that means no repaired damage expressed as the percentage of damage initially induced) may be viewed as indicators of variability in acquired susceptibilities to the genotoxic agents. This marker should express a variation between individuals in vulnerabilities of their DNA to the induction of the damage and the efficiency of the repair process.

## Material and methods

### Sampling

All donors from the reported studies were males, at young to middle age, living in various cities. All donors, at the time of sampling, were reporting themselves as generally healthy, with no apparent symptoms of any disease. The characteristics of investigated groups; genotypes of donors, diet or other factors related to life style, affecting the amount of oxidative type of the DNA damage induced have been discussed elsewhere. There were also reported in details results from monitoring of ambient air pollution, or personal exposures to PAHs [7, 8, 10, 12, 14, 15]. Lymphocytes were isolated from the collected whole blood samples, then frozen according to standard procedures. Frozen samples were transported in dry ice to the laboratory in the Department of Radiation and Environmental Biology at the Institute of Nuclear Physics. Samples were stored at  $-80^{\circ}\text{C}$ . Before studies *in vitro*, lymphocytes were defrosted, viabilities of cells were evaluated with two independent methods. Samples with viability higher than 90% were accepted for further studies [6].

### Challenging treatment and repair competence studies

Cellular capacities were studied immediately after the challenging treatment and after the incubation period during which cells repaired the induced damage [6]. Cells were irradiated with UV-C or X-ray dose from Philips machine model MCN 323 (250 kV). Before and after all irradiation procedures, vials containing cells were kept or transported in the dark, in the box containing ice cubes in water. To avoid repair process during the irradiation procedure, cells were also positioned in the polyethylene box containing ice cubes in water. Irradiated samples were split into smaller parts, in the first one, the DNA damage was at once detected with the SCGE assay, and these results of measures were used to compare susceptibilities to the induction of the DNA damage. The other parts of cells were incubated at  $37^{\circ}\text{C}$  for the time allowing the cells to proceed the repair process, and then residual (no repaired) DNA damage were detected. The half-time of the repair process in irradiated with ionizing radiation lymphocytes of the young healthy male donor estimated from the kinetic studies is  $\sim 5$  min [14]. Therefore, in cases of incubation of X-ray irradiated lymphocytes for the period of about one hour, the amount of residual damage for majority of donors should not decrease anymore.

### DNA damage analysis and detection

The analysis of the DNA damage was done in all groups of cells with the application of the alkaline version of the single cell gel electrophoresis. All chemicals and procedures for SCGE assay were used as described elsewhere [1, 9]. In the studies for the evaluation of the amount of DNA in a comet, an automatic analysis with

the Komet 3.0 software from the Kinetic Imaging Company (Liverpool, UK) was applied. To estimate the levels of DNA damage, two parameters from this software were used:

- tDNA – tail DNA (percentage of the DNA in the comet tail),
- TM – tail moment, the parameter that combines two measures, percentage of DNA in the tail multiplied by the tail length (TL) that is the length of the comet tail measured from the edge of the comet head (calibrated unit of the tail length in our study was 0.862  $\mu\text{m}$ ).

### Standardization and evaluation of cellular capacities

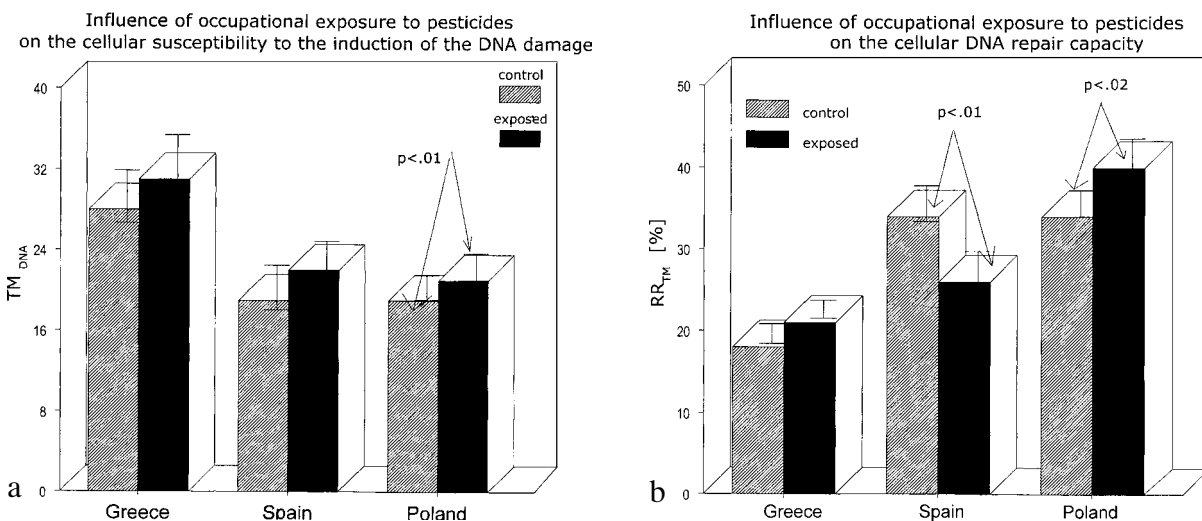
To control the stability of the experimental conditions and to avoid the influence of unpredictable factors on the quality of the final conclusion, an internal standard (IS) was put into operation [6]. Groups of IS cells, belonging to the same pool of cells and the same sampling of lymphocytes from healthy male donor, were subjected in each experiment to the same procedures as the investigated cells. Standardizing factors were evaluated, in respect to each (i) experiment, as the ratio between the tail DNA measures obtained from IS cells responses to the challenging treatment in that particular experiment, and median of IS results obtained from results of all series of experiments in those studies. Experiments in which SF was out of the range (0.65–1.35) were repeated (if cells were available) or excluded.

Efficiency of cellular repair was estimated from the measures of the DNA damage detected immediately after challenging treatment (initial) and remaining as no repaired after the post challenging treatment incubation (residual damage as percent of initial). Repair capacity was evaluated as a percent of the damage repaired. In case of challenging treatment based on ionizing radiation, the repair capacity (RC) is expressed as follows:  $RC_{\text{tDNA}} = [(TDNA_{\text{X-rays}} - TDNA_{\text{X-rays after repair}}) / (TDNA_{\text{X-rays}} - b)] \times 100\%$ , where b – is the background

level of DNA damage calculated from the negative control. The negative control (b value) was also estimated from IS measures, as the value of dispersion of the residual DNA damage detected in “IS” cells in that particular experiment, from the median obtained for the whole series.

### Results and discussion

Human lymphocyte responses to the challenging treatments (X-rays and UV-C exposures) were investigated under the EC project ERBIC 15CT 960300 entitled “Pesticide effects on humans” [7, 8, 14]. Studies were following the general procedure for repair competence assay [6]. Whole blood samples were collected in various countries from 440 healthy donors (controls or occupationally exposed workers to various pesticides in glasshouses). In those studies, two independent exposures (to UV-C and X-rays) were applied for challenging procedures. In Fig. 1a,b results are illustrated (from studies in which exposure to UV-C radiation was applied for the challenging of cells) of cellular capacities obtained for subgroups of unexposed controls and exposed to pesticides glasshouses workers, after stratification according to the origin of the donors. There are observable differences between controls and exposed donors in the average susceptibilities to the induction of the DNA damage, as well as in the evaluated repair capacities. There is also a visible influence of the region. In general, donors from exposed to pesticides groups expressed higher than that of control susceptibility to the challenging UV-C treatment. Lymphocytes of donors exposed to pesticides expressed also a lower cellular DNA repair rate than those of controls, except the Spanish workers (possibly due to differences in social status and diet as it was reported) [8]. A similar decrease in DNA repair efficiency in group of exposed to pesticides donors was observed when irradiation with X-rays was applied in those studies for the challenging of cells, as reported elsewhere [14]. Results of reported



**Fig. 1.** Influence of the occupational exposure to pesticide on cellular susceptibility to the induction of the DNA damage (a) and repair rate evaluated from cellular responses observed in lymphocytes of exposed donors and controls from various countries (b). Repair rate capacity evaluated as  $RRC_{\text{UV}} = [(TM_{\text{UVi}} - TM_{\text{PHA}}) / TM_{\text{UVi}}] \times 100$ , where:  $TM_{\text{PHA}}$  and  $TM_{\text{UVi}}$  were comet tail moments in cells untreated or exposed to UV and incubated with or without a cellular mitogen [8, 14].

**Table 1.** Average for groups from Prague: G=1, Košice: G=2, Sofia: G=3 (number of donors = N) of marker values of exposure obtained from personal monitoring [13] and values of investigated biomarkers of cellular susceptibility, stratified according to the occupational exposure to environmental PAHs (Exp: controls = 0, exposed to PAHs = 1) particular genotype in the investigated group)

G	Exp	N	Age	B[a]P [ng/m <sup>3</sup> ]	Carc. PAHs [ng/m <sup>3</sup> ]	Cotin/ creat <sup>*</sup>	TDNA <sub>0</sub>	TDNA <sub>X-rays</sub>	RD <sub>TDNA</sub> [%]	RC <sub>TDNA</sub> [%]	
1	0	42	mean	29.4	0.9	628	2.6	11.1	25.0	76.5	
			± sd	9.1	0.6	1441	1.4	3.3	11.4	10.8	
	1	48	mean	32.0	1.9	1225	2.7	10.9	36.7	66.0	
			± sd	7.1	1.8	1711	1.2	3.4	21.6	19.6	
			sig.	ns	0.00	0.00	ns	ns	0.002	0.00	
2	0	49	mean	33.6	1.1	3118	4.3	8.2	36.9	65.2	
			± sd	10.1	0.7	4433	1.3	1.5	7.8	8.8	
	1	43	mean	31.7	4.0	3318	4.3	8.0	41.4	60.6	
			± sd	5.2	6.5	4702	1.0	1.5	9.8	10.3	
			sig.	0.00	0.00	ns	ns	ns	0.000	0.00	
3	0	24	mean	39.7	1.4	396	3.7	13.3	33.3	66.7	
			± sd	9.2	0.9	620	1.4	2.1	8.3	7.8	
	1	51	mean	37.0	6.0	1854	3.6	12.8	38.1	61.3	
			± sd	8.7	2.9	3379	1.4	2.4	11.34	8.7	
			sig.	0.01	0.000	0.000	ns	ns	ns	0.002	0.00

\* Cotin/creat = biomarker of smoking pollution (cotine [ng]/creatinine [mg]).

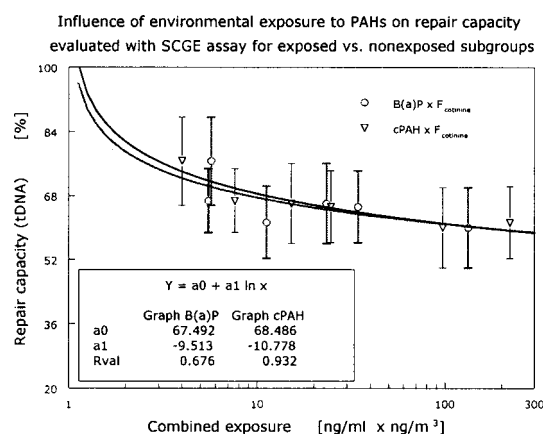
studies, are in good agreement with results of observations on persons occupationally exposed for a long time to pesticides, where alterations of many amino acid metabolism due to exposures were revealed [2]. Results by Anwar have shown that people have diverse responses to exogenous exposures because of variability in the rate of metabolism, DNA repair processes, and factors influenced by environmental or occupational exposure, altering that metabolism. In consequence, exposed populations might have also mutagen-induced abnormal DNA repair responses.

Other groups of donors involving those investigated for possible influence on cellular repair capacities of the occupational exposure to environmental polyaromatic hydrocarbons (PAHs) [10]. Samplings, ambient air and personal monitoring of exposure, and studies on various biomarkers for those group were performed under the EC project EXPAAH and reported elsewhere [10, 15]. A half of the investigated group consisted of young healthy males occupationally exposed to the environmental PAHs, working as city policemen or bus drivers. The other half of the group was matched control, and consisted of males occupationally unexposed to the PAHs. Donors were inhabitants of Prague, Czech Republic, Košice, Slovakia and Sofia, Bulgaria. Information about health and social status of the donors, their education, main habits and life styles were collected through questionnaires. Table 1 presents a brief comparison of investigated groups. There are shown results from personal monitoring and levels of cellular capacities evaluated with repair competence assay. Results are stratified according to various sampling regions and occupational exposure to PAHs [15]. Visible and statistically significant reductions of repair

efficiencies are observed in the groups of donors occupationally exposed to environmental PAHs.

A general confidence exists that polycyclic aromatic hydrocarbons may trigger mutagenesis and subsequent pathological processes, including carcinogenesis. Mutations producing a selectable phenotype in surviving cells may become apparent only in many generations later. 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 contribute greatly in shaping the mutation spectrum of a DNA damaging agent.

In Fig. 2, presented are results of repair efficacy reported in Table 1 as a function of combined exposure



**Fig. 2.** Exposure repair efficacy relationship for bioindicators of combined exposure (i.e. levels of genotoxic agent B[a]P or 8 detected carcinogenic cPAHs evaluated in personal monitoring [15] multiplied, respectively by cotinine level detected in urine that is a biomarker of smoking).

**Table 2.** Influence of EPHX4 genotype, smoking and occupational exposure to environmental PAHs on repair capacity (RC<sub>TDNA</sub>). Average values for groups from Prague: G=1, Košice: G=2, Sofia: G=3. Biomarkers stratified according to genotype, then to smoking habit (NSM – non-smokers, SM – smokers) and then to the exposure to PAHs (PoD percent of donors with that particular genotype in the investigated group)

EPHX4	G	RC <sub>TDNA</sub>	poD [%]	All NSM	NSM control	NSM exposed	All SM	SM control	SM exposed
		all							
Wildtype	1	73.4 ± 16.0	61.8	74.1 ± 16.5	77.3 ± 12.1	69.9 ± 20.6	70.7 ± 16.5	75.8 ± 10.7	67.9 ± 19.2
	2	64.0 ± 10.0	59.2	63.5 ± 9.3	65.0 ± 8.7	62.0 ± 9.7	64.7 ± 10.9	66.9 ± 8.6	62.1 ± 12.7
	3	63.7 ± 9.7	63.0	65.1 ± 9.7	70.6 ± 9.7	61.4 ± 7.8	62.6 ± 9.6	66.0 ± 6.6	60.9 ± 10.4
Heterozygous	1	66.9 ± 17.7	34.8	72.1 ± 14.1	76.1 ± 10.4	66.6 ± 7.3	57.9 ± 20.1	67.7 ± 12.1	56.9 ± 20.9
	2	61.5 ± 9.6	33.3	62.5 ± 8.6	64.2 ± 9.2	60.6 ± 7.6	60.5 ± 10.6	65.6 ± 8.2	55.6 ± 10.6
	3	62.6 ± 6.9	30.1	62.6 ± 7.1	63.0 ± 5.4	62.8 ± 8.2	62.7 ± 6.8	65.2 ± 3.5	61.9 ± 7.40
Homozygous	1	74.6 ± 7.1	3.4	76.0 ± 9.2	82.5 ± 10.4	69.5 ± 7.3	76.3 ± 7.5	76.3 ± 7.5	nd
	2	62.0 ± 9.5	7.5	64.5 ± 8.3	64.3 ± 9.1	64.8 ± 8.3	59.0 ± 10.6	59.7 ± 11.1	57.3 ± 12.1
	3	61.1 ± 7.6	6.9	63.2 ± 6.1	64.3 ± 6.8	61.5 ± 5.4	50.6 ± 5.7	nd	50.6 ± 5.70

[10, 15]. Biomarkers of exposure are presented there in that way that could be considered as indicators of the combined exposures to PAHs resulting from two sources of PAHs; from the ambient air and from cigarette smoking. Factors posing the combined exposure were obtained by multiplying the level of the cotinine detected in urine (biomarker of exposure to cigarette smoke) by the levels of genotoxic agents; benzo-[a]-pyrene (B[a]P) or eighth carcinogenic PAHs (cPAHs) detected in personal monitoring [15]. Results show a clear exposure effect relationship.

Presented results revealed that occupational exposure to environmental polycyclic aromatic hydrocarbons not only significantly reduces cellular repair capacities, but the reduction is the exposure dependent, and exposure effect relationship is more strongly correlated with the exposure to cPAH ( $r = 0.93$ ) than with B[a]P ( $r = 0.68$ ). Visible decreases were also observed when cellular repair efficiency of donors occupationally exposed to environmental PAHs was compared to that obtained for controls. A comparison was done after stratification of the investigated groups, first, according to the polymorphisms in genes encoding enzymes involved in the cellular processes of bio-transformation, and then to smoking and the exposure to PAHs. Table 2 shows an example of variation between the results observed for subgroups of donors characterized by wildtype, heterozygous or homozygous genotype for mutation in gene encoding epoxide hydrolase enzyme (EPHX4), after stratifications first to the subgroups of smokers and non-smokers, and then to exposed to PAHs or controls. Products of EPHX gene catalyze hydrolysis of aromatic epoxides to less reactive trans-dihydrodiols. Hydroxyl groups formed in this reaction are more available for conjugate reactions than epoxides. There are two different alleles of this gene responsible for high and low enzyme activity. Although, genotypes subgroups are less frequent, though on average, stronger reductions of the repair capacities due to smoking and occupational exposure to PAHs are observed in subgroups of donors with heterozygous or homozygous genotypes.

The obtained results show that the occupational exposure to polycyclic aromatic hydrocarbons (PAHs) present as a pollution in ambient air can significantly

reduce the efficiency of DNA repair. A significant decrease of repair efficiency in donors occupationally exposed to environmental PAHs was also observed when the investigated group were stratified, first according to various genotypes for genes encoding enzymes involved in the process of bio-transformation and then into two subgroups: smokers and non-smokers. Observed in those studies, induced by occupational exposure, repair deficiency may be responsible for increase of health risk. Our other reports have shown that such reduction of cellular repair efficacy was observed in groups of donors occupationally exposed to mercury vapors or in cancer patients [7, 12].

Presented results imply that environmental exposures to various genotoxic agents, like various pesticides, polycyclic aromatic hydrocarbons and cigarette smokes, because of the demonstrated possibility of alteration the cellular repair processes can, in consequence, lead to harmful effects hazardous to human health.

## Conclusions

A sensitive and reliable method for the detection of DNA damage or its repair capacity is crucial to many fields from molecular epidemiology and toxicology to preventive and clinical medicine. Data presented in this review indicate that cellular capacities evaluated from responses to challenging dose of radiation and repair competence assay showed dependence on various environmental pollutants, genetic and other factors related to life style of donors. There is evidence that the SCGE results correlate with physical measures and cytogenetic damage that is regarded as a potential good biomarker associated to the alteration of the health risk. Presented results suggest that repair competence assay with the application of the SCGE method might be useful as a predictive assays for susceptibility to various genotoxins and exposures (environmental, occupational, therapeutic).

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