LACK OF CORRELATION BETWEEN DNA REPAIR KINETICS, RESIDUAL DAMAGE AND MICRONUCLEI FREQUENCY IN RADIOADAPTED HUMAN LYMPHOCYTES

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We have previously reported [1] that the kinetics of DNA repair after X-irradiation with 1.5 Gy in radioadapted human lymphocytes differed from that in the non-adapted ones: the repair measured by the comet assay was significantly faster. However, when a larger number of experiments was carried out with cells from other blood donors, this difference was no longer observed, in spite of a clear adaptive response, as revealed by the micronucleus test. The results of these experiments are summarized in two Tables, showing initial and residual damage (Table 1) and parameters of DNA repair kinetics (Table 2). As shown in Tables, no adaptation effect was found also in other experimental groups, where the adapting treatment (irradiation with 1 cGy) was combined with agents that disrupt the transduction of cellular signalling. These were:

- antibody to antigen CD38, the ligation of which initiates tyrosine kinase activity and inhibits the dual enzymatic activities of CD38: adenosine diphosphoribose cyclase and hydrolase;
- TMB-8, antagonist of calcium;
- staurosporine, inhibitor of protein kinases.

Table 1. Initial and residual DNA damage estimated by the comet assay in radioadapted lymphocytes.

Treatment	Tail moment		
	Control ±SD	Initial damage ±SD	Residual damage ±SD
1.5 Gy	4.44 ±3.97*	28.32 ±10.77*	6.23 ±6.38
1 cGy + 1.5 Gy	6.68 ±5.15*	30.96 ±11.36*	7.28 ±6.91
1 cGy + 1.5 Gy	6.73 ±5.36	31.60 ±11.89*	7.22 ±5.81
CD38 +1 cGy + 1.5 Gy	6.26 ±4.15	35.31 ±10.41*	7.83 ±5.25
1 cGy + 1.5 Gy	6.63 ±4.10	36.63 ±10.44*	10.22 ±5.19
TMB-8 + 1 cGy + 1.5 Gy	6.66 ±3.76	29.21 ±13.30*	10.25 ±5.78
1 cGy + 1.5 Gy	6.63 ±4.10	36.63 ±10.44*	10.22 ±5.19
Staurosporine + 1 cGy + 1.5 Gy	6.49 ±4.46	27.74 ±10.29*	9.45 ±5.32

* Significant difference estimated pairwise by Student's t test, $p < 0.05$; SD - standard deviation.

A similar lack of correlation between the effect at the chromosomal (aberrations or micronuclei) and molecular (DNA repair) levels was reported by Wójcik et al. [2].

Table 2. Parameters of DNA repair kinetics*.

Treatment	a ±SD*	b ±SD	c ±SD*	Correlation coefficient
1.5 Gy	22.089 ±4.382	0.108 ±0.067	1.792 ±2.415	0.969
1 cGy + 1.5 Gy	23.674 ±4.448	0.182 ±0.112	0.606 ±1.765	0.978
1.5 Gy	24.380 ±6.080	0.212 ±0.156	0.490 ±0.450	0.967
CD38 + 1 cGy + 1.5 Gy	27.480 ±5.160	0.098 ±0.037	1.560 ±1.100	0.987
1 cGy + 1.5 Gy	26.144 ±5.248	0.239 ±0.136	3.585 ±1.092	0.979
TMB-8 + 1 cGy + 1.5 Gy	18.955 ±7.514	0.223 ±0.071	3.588 ±2.027	0.993
1 cGy + 1.5 Gy	26.144 ±5.248	0.239 ±0.136	3.585 ±1.092	0.979
Staurosporine + 1 cGy + 1.5 Gy	18.293 ±4.97	0.131 ±0.056	2.963 ±0.866	0.986

* The data were fitted to the equation usually used to describe the kinetics of DNA repair: $y=a \cdot \exp(-bt)+c$; SD - standard deviation.

References

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APPLICATION OF THE COMET ASSAY FOR DETECTION OF DNA DAMAGE IN LYMPHOCYTES OF WORKERS EXPOSED TO LOW DOSES OF IONIZING RADIATION

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Detection of DNA damage after chronic exposure to low doses of ionizing radiation presents a practical problem; a suitable technique should be both sensitive and fast. The best biological dosimeter, so far, is based on the determination of chromosomal aberration frequency. This, however, is time-con-

suming and tedious and not suitable for large scale screening. A promising alternative is the comet assay (single cell gel electrophoresis) combined with application of specific enzymes which recognize damaged bases.

Following the lysis and electrophoresis of an undamaged cell one obtains a round nuclear envelope with fluorescent halo formed by undamaged DNA. Cells with damaged DNA give a "comet" consisting of a "head" (nuclear envelope) and a "tail" (DNA attached to the nuclear matrix, but migrating in the electric field). The length of the tail multiplied by the fraction of DNA present in the tail (the so called tail moment) is a measure of DNA damage. Digestion with specific enzymes which recognize damaged bases cause an increase in tail moment.

We examined a group of people occupationally exposed to low doses of ionizing radiation (altogether 49 individuals). Age, smoking habits, use of therapeutic drugs, work-related exposure to hazardous agents, previous exposures to diagnostic X-rays such as patient and nuclear medical examination were registered. For each individual the occupational radiation burden received over the past period of 5 years was adopted from the official personal records based on film dosimetry controlled every month. A matched group of controls was chosen among the administrative employees (40 individuals). The mean age of the studied population at the time of blood sampling was 50 years (range 24-69). The individuals were divided into groups according to risk of exposure, smoking habits and gender. We used non-parametric tests of Mann-Whitney and Kolmogorov-Smirnov for two different samples or Student's test (with correction for separate variance estimation) for comparison of mean tail moments in control and hazard group. We compared the mean tail moments without enzyme treatment, with endonuclease III and FPG (formamidopyrimidine glycosylase) in the tested groups.

There was a significant difference (by both above mentioned non-parametric tests) between the control and hazard groups without enzyme treatment but the level of the oxidative damage was the same in both groups. Higher DNA damage was also found in men than in women. There was no relation of DNA damage to age and smoking habits notwithstanding the enzyme treatment. Additionally, analysis of distributions of tail moment values pointed to a considerable individual diversity even in the control group. Therefore, further investigations are necessary to establish the suitability of the comet assay as biological dosimetry method; the results obtained so far warrant such investigations.

Table. Values of the mean tail moments of all donors compared according to occupational risk, gender and smoking habits.

	Treatment	Tested group		U Mann-Whitney test	Kolmogorov-Smirnov test	t Student test
Risk of exposure		Control group	Hazard group			
No. of people		40	49			
Mean tail moment	**Buffer**	**26.4 ±17.3**	**57.3 ±39.8**	**p = 0.000**	**p < 0.001**	
	ENDO III	99.5 ±40.5	88.4 ±32.6			ns
	FPG	36.9 ±30.1	31.7 ±23.5	ns	ns	
Sex		Women	Men			
No. of people		28	61			
Mean tail moment	**Buffer**	**29.9 ±28.4**	**49.7 ±36.4**	**p = 0.000**	**p < 0.001**	
	ENDO III	105.9 ±36.2	87.7 ±35.6	p = 0.042	ns	
	FPG	**19.9 ±15.4**	**40.4 ±28.3**	**p = 0.002**	**p < 0.001**	
Smoking habits		**Non smokers**	**Smokers**			
No. of people		53	36			
Mean tail moment	Buffer	46.1 ±36.6	39.6 ±33.1	ns	ns	
	ENDO III	89.7 ±36.8	98.9 ±36.2	ns	ns	
	FPG	34.7 ±27.5	32.9 ±25.8	ns	ns	

The work was supported by the European Commission - grant CIPA CT94 0129.

References

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LOVASTATIN TREATMENT DIFFERENTIALLY ALTERS ACTIVATION OF TRANSCRIPTION FACTOR, NFκB AND SENSITIVITY TO HYDROGEN PEROXIDE

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Recently, we undertook an attempt to characterize cellular features that determine sensitivity to hydrogen peroxide of two sublines of murine lymphoma L5178Y [1, 2]. These sublines are inversely cross-sensitive to hydrogen peroxide and X-rays. We found that the amount of initial DNA damage and