

0EFZS--4459

August 1988

BL--749/88

AT 8800371



Österreichisches Forschungszentrum

Seibersdorf

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Lymphocytes of Persons Working at Shlobin
(USSR), 150 Km North of Chernobyl

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OF PERSONS WORKING AT SHLOBIN (USSR),
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Vortrag gehalten beim Internationalen Symposium
"DNA Repair, Chromosome Alterations and Chromatin
Structure under Environmental Pollutions"
Moskau, 4. bis 6. Juli 1988
Proj.Nr. 600016

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INTRODUCTION

SCEs are considered to be a sensitive indicator for the DNA damaging effects of mutagenic and carcinogenic agents. Though the exact mechanism of SCE formation is still unknown, SCE tests are widely used to monitor genotoxic effects of environmental chemicals and occupational exposure. Ionizing radiation per se is not very effective at inducing SCEs (Painter, 1986) compared with UV light or DNA alkylating agents. But in previous studies (Tuschl, 1980) we were able to establish a pronounced effect of low dose exposure on the rate of Mitomycin C (= MMC) induced SCEs in lymphocytes of occupationally exposed persons.

In 1986, during the Chernobyl accident, employees of an Austrian company worked at Shlobin, 150 km north of Chernobyl. At their return to Austria blood was taken and spontaneously occurring and MMC induced SCEs were studied.

METHODS

1. Testpersons

43 male and 2 female persons, with ages varying from 21 to 40 years, were investigated. All testpersons were carefully screened for illness, alcohol consumption and smoking habits. Only obviously healthy persons, without any medication were allocated for the study. According to the inquiry, no alcoholics were among the testgroups. Since all

employees received their meals directly from Austria - starting with 1st May - differences due to different nutrition were minimized.

2. Radiation doses

Since only two of the persons tested carried a personal dosimeter, we cannot give the actual dose for single test persons. Instead, an average exposure dose was calculated for the group of testpersons considered to be "exposed" being present in Shlobin during the Chernobyl accident and staying at least until the end of May. External exposure at the building plot from the 26th to the 30th of April was estimated from a dosimeter brought there by chance already in 1985. Starting with May 1st, 140 personal TL-dosimeters were distributed among workers; in addition the dose rate was measured by a FAG-counter in 1 m height above the ground. The latter decreased from 0.5 mR/h (1st of May) to 100µR/h at the end of May, and 50µR/h at the end of July. For exposed persons of the present investigations the average dose for external exposure from end of April to August was estimated to be about 2 m Sv (see tab. 1).

Table 1: Effective doses at the Austrian building plot in Shlobin (USSR) resulting from external radiation (m Sv).
Estimated dose for 26th April - 30th April: 0.30 m Sv

	lower limit	average	upper limit
May	0.46	0.80	1.26
June	0.24	0.40	0.63
July	0.21	0.30	0.43
August	0.16	0.22	0.28
	1.07	1.72	2.60

Testpersons were divided into two groups: exposed persons - present during the Chernobyl accident and at least till the end of May - and unexposed persons - present in Austria during the accident, and starting their work at the building plot after the 31st of May. By whole-body counting of J131 in probands from Shlobin a committed effective dose equivalent of 0.3 m Sv was calculated for members of the "exposed" groups. In comparison to the exposure due to J131, the burden by incorporation of Cs134 and 137 was very small and could be neglected since it was nearly the same for "exposed" and "unexposed" testpersons.

3. Blood cultures

Whole blood cultures were prepared with 10 ml Gibco RPMI Medium, containing 20% FCS, 0.1 ml Penicillin/Streptomycin (10.000 E, 10µg/ml, Gibco), 0.1 ml PHA (Wellcome), 10µ M/l Bromodeoxyuridine, and 0.6 ml whole blood. For evaluation of induced SCEs, 10^{-7} M/l MMC was used. After incubation of 70 hr in the presence of Bromodeoxyuridine and MMC, 0.1 ml Colcemid (10µg/ml, Gibco) was added. Two hours later cells were centrifuged, treated hypotonically with 0.075 M/l KCl and fixed in 3:1 methanol-acetic acid. Slides were stained by a modified fluorescence-Giemsa technique (Perry, 1974). On each slide at least 20 well spread second cycle mitoses, containing at least 44 Chromosomes, were scored.

STATISTICAL ANALYSIS

A parameter-free analysis of variance according to Kruskal & Wallis was performed to study the effects of exposure and smoking. No analysis was performed on the age of testpersons, since no differences of SCE rates had been demonstrated within the relatively narrow range of 20 to 40 years.

RESULTS

Spontaneously occurring SCEs in lymphocytes of "exposed" and "unexposed" persons were only insignificantly different when results were summarized for all testpersons (tab. 2). A significant influence of smoking could be established (tab. 3). When results were correlated with the time interval between the Chernobyl accident and the date of blood sampling, the increased rate of spontaneous SCEs observed soon after the accident decreased to the normal range during the following months (fig.).

Table 2: Spontaneously occurring SCEs in lymphocytes of "exposed" and "unexposed" persons.

	"exposed" persons	"unexposed" persons
mean	7.7	7.0
standard deviation	± 2.9	± 2.8
number of metaphases scored	460	450
p (5% significance level)	< 0.01%	
mean (of personal means)	7.7	6.9
standard deviation	± 1.2	± 1.4
number of testpersons	23	22
p	> 0.01%, difference not significant	

Table 3: Spontaneously occurring SCEs in lymphocytes of "exposed" and "unexposed" persons; influence of smoking.

	"exposed" groups		"unexposed" group	
	1 non-smokers	2 smokers	3 non-smokers	4 smokers
mean	7.3	7.7	6.5	7.6
standard deviation	± 2.5	± 3.1	± 2.8	± 2.8
number of metaphases	115	285	254	176
p	< 0.01% for 3 < 2, 3 < 4			

MMC induced SCEs were significantly different for both exposure groups (tab. 4, 5), both with and without regard to smoking habits. The effect of radiation exposure showed to be much more pronounced than the effect of smoking.

Table 4: MMC induced SCEs in lymphocytes of "exposed" and "unexposed" persons.

	"exposed" persons	"unexposed" persons
mean	31.6	39.3
standard deviation	± 7.1	± 9.0
number of metaphases scored	406	386
p (5% significance level)	< 0.01%	
mean (of personal means)	31.7	39.6
standard deviation	± 4.0	± 5.7
number of testpersons	20	19
p	< 0.01%,	

Table 5: MMC induced SCEs in lymphocytes of "exposed" and "unexposed" persons; influence of smoking.

	"exposed" groups		"unexposed" group	
	1 non-smokers	2 smokers	3 non-smokers	4 smokers
mean	29.8	31.9	39.2	40.7
standard deviation	± 8.5	± 6.7	± 10.0	± 7.1
number of metaphases	80	269	207	159
p < 0.01% for	1 < 3, 2 < 4			
mean (of personal mean)	29.9	32.0	39.2	40.7
standard deviation	± 6.8	± 3.4	± 6.8	± 3.5
number of testpersons	4	14	10	8
p < 0.01% for	1 < 3, 2 < 4			

DISCUSSION

Previous investigations indicated that chronic low dose radiation had a significant enhancing effect on the DNA repair capacity in lymphocytes of occupationally exposed persons (Tuschl, 1980, Tuschl, 1983). Similar results were obtained in animal studies (Gueth, 1980, Liu, 1987) and had been interpreted as an indication of an error-free inducible DNA repair process. Decreased rates of MMC induced SCEs seem to confirm the assumption that low dose exposure can induce error-free repair. Although the formation of SCEs is still a matter of discussion and controversy, it is generally agreed that they reflect unrepaired DNA damage. Thus radiation exposed persons are obviously able to repair MMC induced DNA lesions to a greater extent than are unexposed controls. This might indicate a hormetic effect of low-level radiation.

Besides an induction of repair enzymes, a shift within lymphocytic subpopulations in favour of more radioresistant cell types must be taken into account. In previous studies we could not find quantitative differences in the ratios of B/T cells between controls and occupationally exposed persons (Tuschl, 1983). Investigations on the rates of Thy 1.2 positive and Lyl 2 positive cells in spleens of C57bl mice irradiated with 1 Gy Co60-gamma radiation showed a complete restoration of subpopulations one week after irradiation (Tuschl, 1986). Nevertheless an extensive study on all lymphocytic subpopulations that can be differentiated by monoclonal antibodies against surface antigens is now carried out to clarify possible effects of low dose radiation on lymphocyte subsets (Tuschl, in prep.).

Irrespective of the molecular mechanism that might be the rationale for the observed decreased induction of SCEs in lymphocytes of exposed persons, the estimation of MMC induced SCEs proved to be the most sensitive test to demonstrate population exposure to low doses of ionizing radiation.

ACKNOWLEDGEMENT - We are greatly indebted to Dr. A. Wottawa for performing the statistical analysis of the results, and Dr. A. Burtscher, Dr. K. Mück, Ing. K. Spalek and Dr. F. Steger for the disposal of the dosimetric data.

This work was supported by the AUVA.

REFERENCES

- Gueth L., Vincze J., Holland J. and Szabo L.D., 1980
"Radiology" (Moscow) **20**, 508-513.
- Liu S.Z., Liu W.k. and Sun Y.B., 1987. "Radiation Hormensis: Its Expression in the Immune System", Health Phys. **52**, 579-583.
- Painter R.B., 1980, "A Replication Model for Sister-Chromatid Exchange", Mutat.Res. **70**, 337-341.
- Perry P. and Wolff S., 1974, "New Giemsa Method for the Differential Staining of Sister Chromatids", Nature **251**, 156-158.
- Tuschl H., Altmann H., Kovac R., Topaloglou A., Egg D. and Günther R., 1980, "Effects of Low Dose Radiation on Repair Processes in Human Lymphocytes", Rad.Res. **81**, 1-9.
- Tuschl H., Kovac R. and Altmann H., 1983, "UDS and SCEs in Lymphocytes of Persons Occupationally Exposed to Low Levels of Ionizing Radiation", Health Phys. **45**, 1-7.
- Tuschl H., Kovac R., 1986, "Subsets of C57bl Mice of Whole Body Irradiation and Application of PAR-Polymerase Modifying Factors". In: Faktoren, die den DNA Metabolismus beeinflussen. Ed.: L. Varga, H. Altmann, Sopron, 1986.

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