XA9745552

IAEA-CN-67/13

LOW DOSE EFFECTS DETECTED BY MICRONUCLEUS ASSAY IN LYMPHOCYTES

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Abstract. The effects of low doses of X-rays between 0.01 and 1 Gy were studied on whole blood samples of various individuals using the cytokinesis-blocked lymphocyte micronucleus assay as an end-point. The adaptive response could be induced in G_0 cells by 0.01 Gy followed by 1 Gy challenging dose within a time period of 8 hours, *in vitro*. The probability distribution of micronucleus increments in those samples which had received very low doses in the range 0.01-0.05 Gy proved to be of asymmetrical type (i.e. lognormal) - very likely to the same shape which has been verified for unirradiated (control) population - while the variable turned to be normally distributed at or above 1 Gy. Profound changes have been experienced in the main characteristics of the linear dose - response relationship and in regression parameters, as well, when successively lessened dose ranges were studied toward 0.01 Gy. In the range below appr. 0.2 Gy the responses were found to be unrelated to the absorbed dose. These findings suggest that in (very) low dose range a higher attention should be needed to biological parameters like repair, protective mechanisms and antioxidant capacities, rather than to the absorbed radiation energy only.

1. Introduction

For the understanding the biological effects of low radiation doses, experimental approaches are needed to reveal biological mechanisms and, furtherly, "epidemiological" survey is advisable to reflect individual responses at selected end-points. In the followings some relevant features of low dose-induced frequencies of micronuclei in human lymphocytes are presented.

2. Materials and methods

The lymphocyte micronucleus assay was performed as described earlier [1,2,3]. X-irradiation of the blood samples taken with lithium-heparin anticoagulant was made at the following conditions: 200 kV_p, 20 mA, 1 mm Cu filter, 60 cm SSD, 0.287 $Gy*min^{-1}$ dose rate, room temperature, 5 ml volume in Falcon plastic flasks. The effects of exogenous apoptosis inducers, mistletoe lectins (ML I, ML II, ML III) were studied on rabbits which had received dose of 1 Gy. After irradiation the animals were treated subcutaneously with various isolectins of 1 ng per kg body weight. Twenty four hours and further 1 and 2 weeks later the micronucleus-carrying cytokinesis-blocked cultured lymphocytes (CB cells) related to the total number of CB cells were scored.

3. Results and discussion

3.1. Adaptive response

The adaptive response could be induced in human lymphocytes of G_0 phase *in vitro* by a conditioning dose of 0.01 Gy and a challenging dose of 1 Gy received by the samples so that a time interval of 1 - 8 hours passed between the two irradiations (*Fig. 1*). The data demonstrate that despite of most of the earlier studies where G_1 cells were conditioned and challenged, the phenomenon of adaptive response could also be observed on G_0 cells in the whole blood. In addition, the micronucleus assay proved to be a proper end-point to study such kind of phenomena. These conditions of investigations even raise the possibility to use this biological response for assessing the individual radiosensitivities. This target was approached by studies which had been made on the radiation-induced individual increments of micronucleus frequencies in a population.

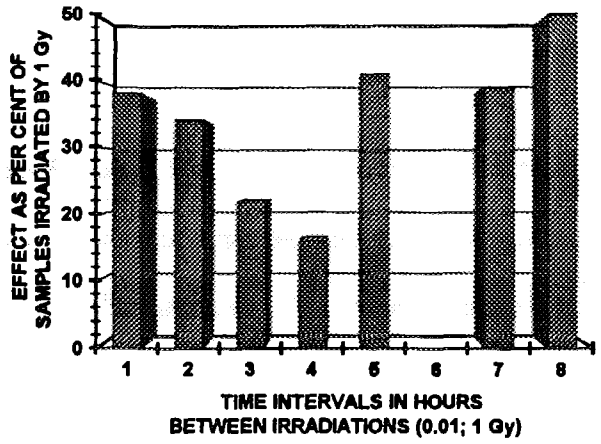

Figure 1. Adaptive response of G_0 human lymphocytes X-irradiated in whole blood, in vitro.

3.2. Individual differences in radiation-induced increments of micronuclei

The frequency in all individual samples reacted with a well observable increase against the self-control value, even at 0.01 Gy, as it has been proved by the *Friedman* analysis of variance test by ranks [3]. Profound alterations were observed in the shape of probability distribution of increments at (very) low or high doses. In samples which had received (very) low doses, the variable of increment seemed to be asymmetrically distributed: the corresponding lognormal frequency function could be fitted with far the best approximation (*Fig 2*). This type of probability distribution was found to be identical with that which had already been proved for non-irradiated control population, too.

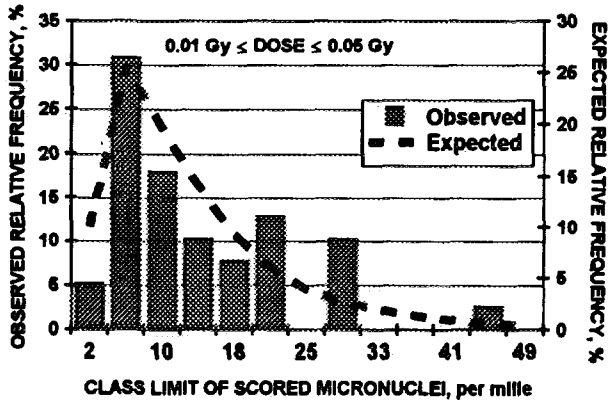

Figure 2. Frequency histograms of the observed micronucleus increments induced by very low doses of 200 kV_p X-rays and the density function of the corresponding lognormal distribution. The lognormal approximation for increments was accepted. [Calculated statistics: $\chi 2(df)=1.751(4)$; $p=0.781$; $M(raw)=13$; $M(ln)=2.3$; $SD(raw)=9.6$; $SD(ln)=0.8$; $m_e(raw)=10$; mode=6; frqucy of mode=7; skewness=1.168; curtosis=1.205]

At higher dose, i.e. 1 Gy, the pattern of distribution took a different turn: the occurrence of micronucleus increment proved to be consistent merely with the corresponding normal curve (*Fig. 3*). These findings suggest different biological protective mechanisms determining the responses. At low doses probably the individual antioxidant capacities in sera might modulate the response, while at higher doses, after the exhaustion of the antioxidant potential, the repair processes might play a decisive role in development and manifestation of an end-point.

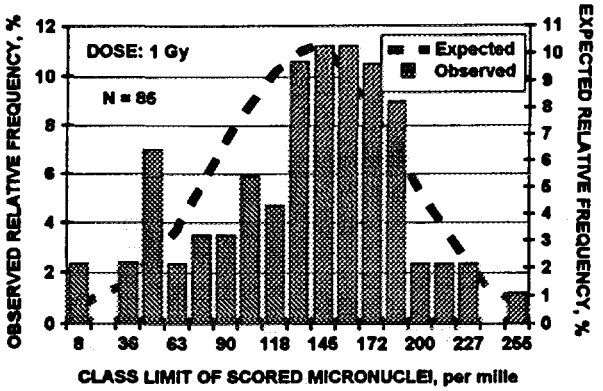

Figure 3. Frequency distribution of the observed micronucleus increments induced by 1 Gy of 200 kV_p X-rays and the expected normal curve constructed by the sample parameters. There was sufficient reason to accept the consistency. [Calculated statistics: $\chi 2(df)=15.927(10)$; $p=0.102$; $M=134$; $SD=53$; $m_e=142$; mode=166; frqucy of mode=6; skewness=-0.511; curtosis=-0.299]

3.3. Main characteristics of the linear dose - response equation

A detailed regression analysis, including that of residuals, was accomplished on the initial part of the dose - response curve. Thus, all the significant changes regarding both the correlation coefficient and slope and the proportion of variance of increment due to the dose could be revealed. Interestingly enough, when the dose range 0.01 - 1 Gy was successively cut toward the lower limit of data, in the interval *below* 0.18 Gy the slope started to "disappear" and, simultaneously, the random component of the variance became of considerable importance. In other words, the micronucleus increment was found to be unrelated to the absorbed dose in the dose range in question (*Table 1*). These results suggest that biological response-modifying factors might play more decisive role than the absorbed energy alone. The phenomenon might also explain differences in the individual adaptive response capabilities as compared with the conditioning doses in this interval.

Table I. Summary of the main parameter values and characteristics of linear dose - response equation.

Parameter	*Dose interval, Gy*						
	0.01-1	*0.01-0.7*	*0.01-0.3*	*0.01-0.22*	*0.01-0.18*	*0.01-0.13*	*0.01-0.1*
N	346	261	210	143	126	108	88
Slope, *b*	127.38	42.27	36.88	25.17	14.20	- 1.99	- 12.42
±SE, *b*	4.73	3.36	5.68	8.65	11.75	16.56	22.01
p	0.000	0.000	0.000	0.004	0.229	0.904	0.574
R	0.824	0.616	0.411	0.238	0.108	0.012	0.061
Proportion of variance of *Y* (incr) due to *X* (dose)	0.678	0.380	0.169	0.057	0.012	0.0001	0.004
No. of outliers > (±2SD), %	*Obsrvd: 7* Expctd: 5	*4* 5	*4* 5	*4* 4	*5* 5	*4* 4	*5* 5

3.4. Induction of apoptosis in radiation-damage carrying cells

Relatively low doses influence the structure and function of cell membranes [4]. In fact, differences in signal transfer leading to apoptosis between the normal and the radiation-damage carrying cells were demonstrated as the latter being more sensitive against the exogenous apoptosis inducer mistletoe lectins than the former (*Fig. 4*). This novel effect of the mistletoe lectins suggest that a scavenging mechanism is inducable. The preferential elimination of injured cells might support the efficiency of radiotherapy

allowing the reduction of treatment dose as well as the "cleaning-up" of the organism from aberrant cells. These occurrences might reduce the risks of late pathological processes.

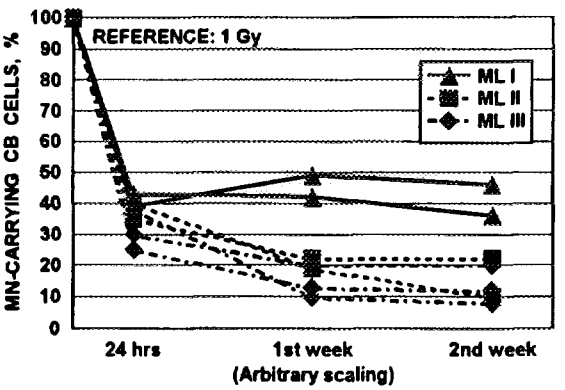

Figure 4. Reduction of radiation-induced micronucleus-carrying CB cells upon treatment of rabbits in vivo *with mistletoe lectins.*

4. Conclusions

In the (very) low dose range, as a rule, biological effects are modulated rather by biological factors or conditions than by the absorbed dose. Using cytogenetic end-points either for occupational or for accidental dose assessments, the calibration below approximately 0.2 Gy has an inherent uncertainty reflecting also the individual sensitivities. At the same time, in this dose range the biological support like the increase of antioxidant capacities and promoting the repair processes of cells and the organism might provide efficient protection. The responses in the dose range in question might be characterized by "non-linear, non-threshold" feature. Radiation-injured cells presumably carry such " *stigma*" which render them susceptible to a scavening mechanisms through apoptosis provoked either by exogenous lectins as it has been demonstrated, or any other endogenous factors. The observations and approaches presented here contribute to the understanding low dose effects and also emphasize the need of further investigations at the level of experimental cellular radiation biology with particular reference to individual reactions in human population.

Acknowledgements. The authors are indebted for their collaborations to Professor U. Pfüller, Univ. Herdecke/Witten, Germany and to Dr. M. Ótós, Atommed Ltd. Paks, Hungary. The technical assistances of Ms. E. Tóth and Ms. M. Nagy are acknowledged. This work was supported by Ministry of Public Welfare No. 08-014/1996 and EC PECO No. ERBCIPDCT 940224.

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