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Original Contribution

THE MICRONUCLEI FREQUENCY AS A BIOLOGICAL DOSIMETER OF ABSORBED DOSE IN CASE OF RADIATION ACCIDENTS

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ABSTRACT

The scoring of micronuclei in human peripheral blood lymphocytes is used as a biomarker and dosimeter of radiation exposure. In this paper we investigated a dose response curve for micronuclei in human lymphocytes following Cs-137 irradiation in vitro. The lymphocytes were obtained from 7 different donors aged between 24 and 51 years. The applied doses were: 0.0, 0.05, 0.1, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0 and 4.0 Gy. There was an increase of micronuclei frequency MN with the dose. The dose-effect relationship was expressed with the following linear-quadratic equation $Y = 20{,}56 + 39{,}59.D + 17{,}01.D^2$, where (Y)-represents the micronuclei yield, (D)-represents the applied radiation dose. A software program for absorbed dose assessment based on this equation was created.

Key words: biological dosimetry, micronuclei, gamma-rays, radiation accidents

INTRODUCTION

Biological dosimetry is an important part of diagnosis and prognosis in case of overexposure suspicious to ionizing radiation. It is very important to estimate the dose absorbed by the exposed persons in order to plan their therapy. Even when the physical measurements of the dose are feasible, independent dosimetry by biological methods can prove to be very useful.

Currently the most used biological dosimeter of exposure to ionizing radiation is the frequency of chromosomal aberrations in peripheral blood lymphocytes. Many types of chromosomal aberrations may appear in lymphocytes following exposure to radiation, but dicentric chromosome is a gold biomarker for ionizing radiation exposure and is proved to be a biodosimeter for estimating the radiation dose (1-4). The application of the conventional metaphase analysis takes long time and well-trained staff is needed. Moreover, after a radiation accident, there is an urgent need to determine the absorbed dose as quickly as possible.

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The cytokinesis-blocked micronucleus technique has the potential of being a simple and fast method of biological dosimetry. It has been applied successfully in vitro to human peripheral blood lymphocytes in 1985 by Fenech and Morlay (5). The yield of induced micronuclei (MN) was assessed by inhibiting cytokinesis by cytochalasin-B. A lagging chromosome fragments or whole chromosome that is unable to interact with the spindle form the micronuclei. These micronuclei appear as small spots in the interphase cells that have undergone one cell division (2, 6). They are scored in binucleated cells with intact cytoplasm (2, 6). The micronucleus test is simple, rapid and cheap. It has the potential for automation (7). It is important and suitable for biological dosimetry in case of radiation accidents. The confounding factors which may influence the results are age, gender, smoking (8, 9).

In this paper we present data on the in vitro dose-response curve applying citochalasin-B blocked micronucleus assay for absorbed dose assessment in case of radiation accidents. We studied the dose-effect relationship of micronuclei yields in the range from 0.00 to 4.00 Gy after gamma-irradiation.

MATERIALS AND METHODS

Sample collection and irradiation

Blood samples were taken by venipuncture in heparinised vials from healthy donors (3 male and 4 female) with no radiation working history to establish in vitro dose-response curve. Their ages ranged from 24 to 51 years. The samples were transported immediately after the collection to the laboratory. 3 ml of each sample was placed in a glass flask for irradiation. The blood samples were irradiated in the flasks on ice with doses of 0; 0.05; 0.1; 0.25; 0.5; 0.75; 1; 1.5; 2; 3; 4 Gy. All irradiations were performed in the National Center of Radiaobiology and Radiation Protection (NCRRP) on Cs-137 gamma-rays machine. The doses were applied at 0,63 Gy/min dose-rate.

MN culture

After irradiation, duplicate whole blood cultures per subject were established by adding 1ml heparinised whole blood into 9 ml of RPMI-1640 medium containing heat inactivated normal calf serum, antibiotics and phytohaemaglutinin (PHA) for initiation of the cultures. The blood cultures were incubated in CO_2 incubator at 37 °C. At 44h after stimulation with PHA the cytochalasin B in concentration of 0,6 μg/ml was added to the culture medium to block a maximum of lymphocytes at the beginning of the second interphase. The cells were harvested at 72h. The contents in the flasks were centrifuged and the sediment was treated with 0.075 mol/L KCl. After the fast hypotonic treatment the cells were fixed with freshly prepared fixative solution of methanol/acetic acid (3:1). After two times with the fixative, cell sediment was finally resuspended in small amount of the fixative and mixed gentle. This cell suspension was dropped onto microscope slides and left to air-dry at room temperature. Slides were stained with 5% Giemsa solution. All slides were coded and scored blindly. Briefly 2000 binucleated cells (CB) per subject were scored for the presence of micronuclei. Only binucleated cells were considered for scoring.

Statistics

The statistical analysis was performed by SPSS 11.0.1. program. The results are presented by means of standard deviation.

RESULTS AND DISCUSSION

The dose-response data obtain by scoring cytokinesis-blocked cells of 7 donors, as well as the intercellular distribution of micronuclei at different radiation doses are given in **Table 1**. An enhancement in micronuclei and cells with micronuclei with increasing of the dose was observed.

Table 1: *Title: Micronuclei frequencies and distributions in lymphocytes from seven donors irradiated in vitro with different doses gamma rays.*

Dose (Gy)	Total cells scored, Mean ± STDEV	Total cells with micronuclei Mean ± STDEV	Micronucleus distribution			Cells with micronuclei/ 1000 cells	MN/1000 cells
			MN1 Mean ± STDEV	MN2 Mean ± STDEV	MN⟩3 Mean ± STDEV	Mean ± STDEV	Mean ± STDEV
0	2000	40 ± 17	36 ± 14	4 ± 3	0	20 ± 8	22 ± 10
0.05	1935 ± 170	42 ± 20	37 ± 17	4 ± 3	0	21± 9	23± 11
0.1	2000	54 ± 21	49 ± 18	4 ± 3	1 ± 1	27± 11	29 ±12
0.25	2000	56 ± 9	51± 10	5 ± 2	1 ± 1	28 ±5	31± 5
0.5	2000	77 ± 11	70 ± 9	5 ±2	1 ± 1	38± 5	38± 5
0.75	2000	108 ± 36	100 ± 28	8 ± 7	1 ± 1	54 ±18	54± 18
1	2000	130 ± 31	119 ± 30	10 ± 3	1 ± 1	65 ±15	65± 15
1.5	2000	198 ± 61	173 ± 47	22 ± 13	4 ± 3	99 ±30	99 ±30
2	1857	278 ± 90	241 ±72	31 ± 17	5 ± 3	153± 45	153± 45
3	1714 ± 487	426 ± 186	342 ± 136	73 ± 43	15 ± 12	241± 58	241 ±58
4	2000	727 ± 155	547 ± 77	131 ± 58	31 ± 20	363± 78	449 ±116

The yield of micronuclei at 0,00 Gy dose which relates to the natural background is 22 per 1000 CB. The spontaneous micronuclei frequency is shown to be variable (2, 4, 5). In literature values between 2 to 36 per 1000 CB are indicated (2, 4, 5). Our data are compatible with this range. The micronuclei frequency and the number of cells containing micronuclei show the following variability for our control subjects, from 16,5 to 41,5 per 1000 CB and from 11 to 24,5 per 1000 CB, respectively. Spontaneous frequency and variability make the method inaccurate for biological dosimetry at low dose exposures. Because of this, in our work we have 5 dose points at low doses between control and 1.00 Gy dose range at which most of the possible radiation accidents occur (10). Some authors observe an increase of micronuclei frequency with the age of the donors (8). The correlation in background micronuclei and micronucleated cells frequency with donor age, smoking habits and gender is not observed in our study ($p > 0.05$).
The curve of the induced micronuclei as a function of radiation dose is shown in **Figure 1.**

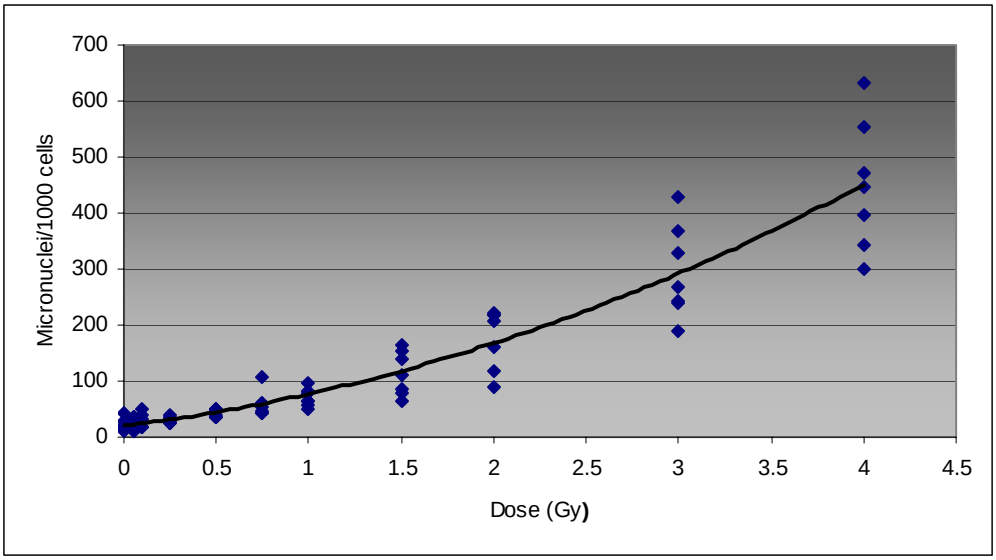

Figure 1: *Title: Dose-response curve of micronuclei frequency in human peripheral blood lymphocytes in vitro irradiated with different doses gamma rays.*

The figure presents an increase of micronuclei yield with the dose in linear-quadratic way. The dose-effect relationship was expressed with the following linear-quadratic model:

$$Y = 20,56 + 39,59.D + 17,01.D^2$$

where Y is micronuclei yield, D is the radiation dose (Gy).
The observed α and β coefficients for the induction of micronuclei are calculated by Nonlinear Regression analysis of SPSS-version 11.0.1 statistical program.
It can be noted that α coefficient is two times greater compared to the β value. The presence of the β value in the MN response curve shows the existence of indirect effects and two track mechanisms in creating the chromosomal damage leading to expression of the micronuclei. The α/β ratio of MN yield in our study was 2,33 Gy representing the dose at which both single-track and two track events are responsible from chromosomal damage equally. This ratio shows that our results are compatible with those in the literature (11, 12) and the linear slope of the curve is more pronounced than the quadratic one at doses up to 5 Gy with low linear energy transfer (LET).The linear and quadratic coefficients, estimated on the dicentric analysis for the dose range 0.0-5.0 Gy present greater pronounced quadratic component (1, 4, 10). This component is a result of chromosome aberrations formed by two-track events, which are mostly responsible for the aberrations at high doses (1, 4, 10). Based on the excess acentric yield, the data are in agreement with our results, two times more pronounced linear component (1). Equal α and β components were observed in our previous data on dose-

response relationship for conventional dicentric analysis, created after gamma exposure for 0.0-3.0 Gy dose range (13).
It is also very important to note that the determination coefficient value is high (R^2= 0.8928). This shows that 89% of the cells with micronuclei are produced by radiation exposure.

For biological dosimetry purposes a software program has been created for absorbed dose assessment, based on our equation and the coefficient values.
Table 2 presents α and β values, fitted from in vitro dose-response models for micronuclei frequency by different authors (4, 11, 12, 14).

Table 2: *α and β coefficient values of in vitro dose-response models for micronuclei frequency reported by different authors.*

Authors	α coefficient ±SD	β coefficient ±SD
Prosser J et al (4)	0.117± 0.006	0.0087 ± 0.0016
Bhat&Rao (11)	0.110 ± 0.026	0.041 ± 0.002
Ban S. et al (14)	0.087 ± 0.010	0.009 ± 0.004
Huber et al (12)	$0.51x10^{-1}$± 0.10	$4.8x10^{-2}$ ± 1.3

It is observed that the linear coefficient is more pronounced than the quadratic one (4, 11, 12, 14). This is in agreement with our results.

CONCLUSION

The micronucleus method has become a possible alternative analysis than the conventional dicentric methods for radiation dose assessment in case of radiation accidents. It is faster, simpler and easier. In this paper we have established a dose-response curve of the radiation induced micronuclei. The frequency of micronuclei in irradiated human lymphocytes is dose dependent, and therefore, can be used as a biological dosimeter. The micronucleus test also showed some limitations of the method especially at low dose levels, because of the variation observed. A reasonable solution could be the supplementation of the conventional micronucleus assay with fluorescence labelled pan-centromeric probe (15). This approach is in progress in our biological dosimetry laboratory.

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