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Individual variations in the micronucleus assay for biological dosimetry after high dose exposure

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ABSTRACT

The micronucleus assay is widely used as a biological dosimeter. Due to an inhibitory effect of radiation on cell proliferation the assay yields satisfactory results only when the absorbed dose is below about 5 Gy. In 2002 Müller and Rode suggested that a modified version of the test, based on the analysis of the ratio of trinucleated to tetranucleated cells and the frequency of micronuclei (Mn) in binucleated cells containing at least one Mn, can be applied to detect a dose reaching 15 Gy (Mutat. Res. 502 (2002) 47–51). Their conclusion was based on the results of experiments with lymphocytes from one donor and nothing is known about the possible influence of individual variability on the applicability of the Mn test to detect high doses of radiation. The aim of the present study was to validate the modified micronucleus assay with lymphocytes of 5 donors. Their blood was exposed to 0, 5, 10, 15 and 20 Gy of ^{60}Co gamma rays. The levels of Mn and of cell proliferation were assessed using various approaches. A strong inter-individual variability was observed for all endpoints. The results clearly show that the assessment of cell proliferation is essential for the interpretation of results. Unfortunately, it was not possible to identify one single proliferation marker that gives all necessary information.

1. Introduction

An important endpoint of biological dosimetry is the analysis of cytogenetic damage (chromosomal aberrations and micronuclei) in peripheral blood lymphocytes (PBL) [1]. In order to visualise the cytogenetic damage PBL must be stimulated to proliferate in culture. However, the ability to proliferate is inhibited by radiation-induced damage to the DNA [2,3]. This inhibition probably affects the most seriously damaged cells. Consequently, the dose–response curve for chromosomal aberrations or micronuclei tends to level off at doses exceeding 5 Gy [4]. It should be mentioned that this effect is not restricted to radiation but applies also to chemical mutagens [5].

One method to circumvent the problem of proliferation inhibition is the induction of premature chromosome condensation (PCC) [2,6,7] and this technique has been successfully applied to detect the doses absorbed during the Tokaimura radiation accident [8]. However, PCC is not widely established, perhaps due to the high skill required for the analysis of aberrations. In contrast, a simple and widely applied assay is the micronucleus (Mn) test [9–11]. Müller and Rode suggested that a modified version of the test can be applied to detect a dose of X-radiation reaching 15 Gy [4]. The major modification involves the analysis of the ratio of trinucleated to tetranucleated cells and the frequency of Mn in binucleated cells containing at least one Mn.

A problem associated with the Mn test is the relatively large inter-individual variability with respect to the shape of the dose–response-curve for Mn [12–15]. In their experiments Müller and Rode analysed PBL of only one donor and nothing is known about the possible influence of individual variability on the applicability of the Mn test to detect high doses of radiation. The present investigation was undertaken to clarify this point.

Blood of 5 donors was exposed to 0, 5, 10, 15 and 20 Gy of ^{60}Co gamma rays. The levels of Mn and of cell proliferation

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Table 1
Distributions and frequencies Mn as well as proliferation markers of lymphocytes per dose and donor. BNC: binucleated cell; DI: dispersion index; MnBNC: micronucleus containing BNC; MNC: MNC, BNC, TriNC, TetNC; PenNC: 1–5 nucleated cells respectively.

Donor	Dose (Gy)	Number of BNC with					BNC scored	DI	Mn per 1000 BNC	Number of MnBNC	Mn per MnBNC	Number of					Cells scored
		0 Mn	1 Mn	2 Mn	3 Mn	4 Mn						MNC	BNC	TriNC	TetNC	PenNC	
1	0	996	4	0	0	0	1000	0.99	4	4	1.00	260	226	3	9	2	500
	5	359	146	66	29	7	607	1.35[a]	647	248	1.58	390	54	2	1		447
	10	128	25	13	27	9	202	1.94[a]	832	74	2.27	473	27	5	2		507
	15	314	3	4	1	1	323	2.39[a]	56	9	2.00	487	12	3	1		503
	20	301	2	2	1	0	306	2.09[a]	29	5	1.80	461	37	2			500
2	0	999	1	0	0	0	1000	1.00	1	1	1.00	312	174	7	7	0	500
	5	481	79	9	3	0	572	1.16[a]	185	91	1.16	419	66	4	1	1	491
	10	234	39	20	6	0	299	1.46[a]	324	65	1.49	411	59	10	3	4	487
	15	152	28	9	1	0	190	1.24[a]	258	38	1.28	445	44	2	2	0	493
	20	124	18	5	0	0	147	1.18	190	23	1.21	452	41	2	2	2	499
3	0	999	1	0	0	0	1000	1.00	1	1	1.00	201	285	7	7	0	500
	5	848	101	15	3	2	969	1.34[a]	153	121	1.22	407	77	5	1	1	491
	10	346	96	52	11	2	507	1.33[a]	475	161	1.49	413	69	12	4	2	500
	15	273	47	28	22	5	375	1.18[a]	504	102	1.85	431	65	4	0	0	500
	20	211	17	5	3	0	236	1.63[a]	153	25	1.44	442	49	7	1	1	500
4	0	997	3	0	0	0	1000	0.99	3	3	1.00	371	103	13	10	3	500
	5	479	177	98	29	11	794	1.36[a]	635	315	1.60	392	52	11	6	3	464
	10	358	35	28	23	23	467	2.33[a]	540	109	2.31	417	64	4	1	0	486
	15	273	2	3	5	1	284	2.69[a]	95	11	2.45	415	71	8	5	1	500
	20	212	7	1	1	2	223	2.51[a]	90	11	1.81	457	39	2	2	0	500
5	0	996	4	0	0	0	1000	0.99	4	4	1.00	303	181	3	1	0	488
	5	228	63	19	1	0	311	1.09	334	83	1.25	394	101	3	1	1	500
	10	204	14	4	2	1	225	1.87[a]	142	21	1.52	412	84	3	1	0	500
	15	121	9	5	1	1	137	1.90[a]	190	16	1.62	464	34	2	0	0	500
	20	156	0	2	1	0	159	2.40[a]	44	3	2.33	420	37	7	3	0	467

[a] DI value significantly overdispersed.

were assessed using various approaches. A strong inter-individual variability was observed for all endpoints. The results clearly show that the assessment of cell proliferation is essential for the interpretation of results. Unfortunately, it was not possible to identify one single proliferation marker that gives all necessary information.

2. Materials and methods

2.1. Irradiation, lymphocyte culture and slide preparation

The experiments were performed on peripheral blood obtained through venipuncture from five healthy donors (3 females and 2 males) aged between 23 and 26 years. 10 ml of blood per donor was collected on two occasions, first from 3 and subsequently from 2 donors. Shortly after collection, 1.5 ml of blood from each donor was aliquoted into 1.5 ml Eppendorf tubes, transported to the Swietokrzyskie Oncology Center and exposed to 0, 5, 10, 15 and 20 Gy of gamma rays at a dose rate of about 1 Gy/min (^{60}Co Theratron Elite 80 source). Transport of blood and irradiation was performed at room temperature. The transport time was about 1 h. The Eppendorf tubes were placed in paraffin boluses to allow for electron equilibrium. In order to reduce the total time of exposure the doses were applied in fractions of 5 Gy, whereby tubes were successively removed from the beam. The interruption in dose delivery lasted less than 1 min. Following exposure the blood was transported back to the laboratory where 0.5 ml of blood was suspended in complete medium which consisted of: 80% RPMI 1640 (Sigma); 20% foetal calf serum (FCS, Sigma); 15 μl/ml phytohaemagglutinin (PHA, GIBCO M-form); 5 μl/ml antibiotic/antimycotic (GIBCO). Single cultures per donor and dose were set up. All cultures were incubated at 37 °C in 5% CO_2 for 72 h. Cytochalasin B (Sigma; final concentration: 5 μg/ml) was added after 44 h of culture time and left for the final 28 h until harvest. Cells were harvested as described in Ref. [16] and dropped on clean slides and stained with Giemsa on the following day.

2.2. Analysis of micronuclei

The frequency of Mn was estimated by analysing a maximum of 1000 binucleated lymphocytes (for each dose and donor) on coded slides. The actual numbers of analysed cells are given in Table 1. A maximum of 500 cells were scored for calculating the replication index (Table 1). Analysis was performed under a 400× magnification.

2.3. Statistical analysis

The distribution of Mn per BNC was analysed by calculating the dispersion index which is the product of the variance divided by the mean Mn/BNC. The significance of deviation from a Poisson distribution was tested with the *U*-test as described in Ref. [17]. The replication index (RI) was calculated as previously [18] according to the equation:

$$\mathrm{RI} = \frac{\mathrm{MNC} + (\mathrm{BNC} \times 2) + (\mathrm{TriNC} \times 3) + (\mathrm{TetraNC} \times 4)}{N} \quad (1)$$

where MNC, BNC, TriNC and TetraNC indicate mono-, bi-, tri- and tetranucleated cells, respectively and *N* the sum of scored cells.

3. Results

Numerical results are shown in Table 1. The individual dose–response curves for the frequency of Mn per BNC are shown in Fig. 1A and for Mn per micronucleated BNC (a BNC with at least one Mn) are shown in Fig. 1B. A somewhat lower degree of individual variability was observed for Mn per micronucleated BNC than for Mn per BNC. Also, the mean frequency of Mn per micronucleated BNC increased until a dose of 10 Gy and showed a lower decline at 15 and 20 Gy than is the case for Mn per BNC. The distribution of Mn per BNC tended to deviate from a Poisson distribution with increasing dose of radiation (Table 1).

The dose–response curves for the percent of MNC and BNC are shown in Fig. 2A and B. As expected, the percent level of MNC increased with dose and the percent level of BNC decreased with dose. Interestingly, in the case of donor 1 and donor 5, the percent of MNC was lower and that of BNC higher after 20 Gy than after 15 Gy.

The replication indices are shown in Fig. 3. In accordance with the data shown in Fig. 2 the RI tend to decrease with increasing dose. The largest decline was observed between 0 and 5 Gy.