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Fundamental and Molecular Mechanisms of Mutagenesis

Radiosensitivity of blood lymphocytes from basocellular carcinoma patients, as detected by the micronucleus assay

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Abstract

Cytogenetic techniques, the micronucleus (MN) assay, in particular, have been widely used in population monitoring, biological dosimetry and early detection of groups susceptible to cancer. Individuals respond differently to several environmental agents. The efficiency of the cellular repair mechanisms would be responsible, at least to some extent, for individual differences in sensitivity to neoplasia. In order to determine the sensitivity of cancer patients to ionizing radiation, blood cultures from untreated individuals with basocellular carcinoma, young healthy subjects and older healthy subjects, were irradiated in vitro with ^{60}Co at doses ranging from 0 to 500 cGy and submitted to the cyto-B micronucleus assay; the frequency of cells and distribution of MN and dose–response relationships were analyzed. Results showed that cancer patients had a lower frequency of cells with spontaneous MN than older healthy subjects. The frequency of micronucleated cells was not different in patients and healthy subjects, but not the distribution of MN per radiation dose: for the carcinoma group, while the proportion of cells with one MN decreases drastically, the proportion of the cells with two or more MN increases with the same intensity. Our results show that the proportion of damaged cells is similar in patients with basocellular carcinoma and healthy subjects, but the magnitude of radiation-induced lesion is greater in the cancer patients.

Keywords: Micronucleus; Gamma radiation; Human lymphocyte; Basocellular carcinoma; Radiosensitivity

1. Introduction

Epidemiological studies show that not all individuals similarly exposed to genotoxic agents develop neoplasia. This variability in response may result from different degrees of efficiency of the cellular repair mechanisms (Hsu et al., 1989) and from individual susceptibilities to several kinds of mutagens, such as clastogenic, radiomimetic, alkylating and dimer inducing agents. The deficiency in a type of repair mechanism may be more evident for a given mutagen than for another (Hsu et al., 1985).

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Susceptible individuals may accumulate more mutations and chromosome damage than resistant ones, being therefore more prone to the development of neoplasia (Setlow, 1978).

Since genetic alterations are intimately associated with the development of cancer, the quantification of lesions occurring in tissues may serve as an indicator of carcinogenic risk. Among several techniques for detecting DNA damage, the micronucleus test has proved to be quick, efficient and very sensitive. Hence, it has been used in monitoring, biological dosimetry and early detection of groups at risk for developing cancer, including individuals occupationally or accidentally exposed to carcinogens (Högstedt, 1984; Barregard et al., 1991; da Cruz et al., 1994), with premalignant lesions (Rosin et al., 1994) or with a family history of increased incidence of cancer (Bondy et al., 1993), smokers (Sarto et al., 1990; Tomanin et al., 1991) and alcoholics (Stich and Rosin, 1983). In these cases, a significantly higher frequency of chromosome breaks was observed when compared to healthy subjects.

Investigations on chromosome fragility in patients with cancer may contribute to a better understanding of the role of susceptibility to mutagens in environmental carcinogenesis.

One way of detecting genetic instability in individuals is through the induction of chromosome breaks by mutagenic agents (Hsu et al., 1989). Patients with different types of cancer show a significantly higher incidence of bleomycin-induced chromatid breaks, suggesting the presence of some deficiency in DNA repair mechanisms (Hsu et al., 1989; Schantz and Hsu, 1989).

Fibroblasts from melanoma patients show higher frequencies both of spontaneous and UV-induced MN when compared to healthy subjects (Roser et al., 1989). Lymphocytes from leukemia patients irradiated in vitro and in vivo with X-rays do not differ significantly from controls in the spontaneous frequencies of binucleated cells with MN (Gantemberg et al., 1991).

The purpose of this work was to evaluate the chromosome susceptibility to ionizing radiation of individuals with basocellular carcinoma in comparison to healthy subjects, using the micronucleus assay.

2. Materials and methods

2.1. Blood donors

Blood samples were obtained from 3 patients (one white male, 68 years old; two white females, 47 and 67 years old) with facial basocellular carcinoma, diagnosed at the Dermatology Clinic of the Santa Casa Hospital, São Paulo, SP, Brazil, not having received any chemical or radiation treatment before the blood was collected (group C).

Two control groups consisted, respectively, of 4 white healthy young individuals (two males, aged 24 and 27 years, and two females, aged 20 and 27 years) (group A) and two healthy older individuals (one white male, aged 50 years, and one Japanese female, aged 46 years) (group B). Controls (groups A and B) were non-smokers, with no history of exposure to radiation, had not consumed alcohol or any other drug and had no symptoms of any disease at the time the blood sample was taken.

All subjects answered a questionnaire to provide additional information on personal habits, life style, exposure to known mutagenic agents, etc.

2.2. Irradiation

Blood samples (5 ml) from each donor were obtained using heparinized syringes and needles. They were divided into 8 lots and irradiated in a ^{60}Co source (Gammacell 220, Irradiation Unit of Canadian Atomic Energy Commission, Ltd.) with doses of 0, 20, 50, 100, 200, 300, 400 and 500 cGy at an average dose rate of 1 Gy/min.

2.3. Lymphocyte cultures

Blood samples were prepared for cytochalasin-B cytokinesis-block MN assay (Fenech and Morley, 1985) with some modifications: 0.5 ml of whole blood was added to 4 ml of RPMI-1640 medium (Cultilab), 20% bovine fetal serum (Cultilab) and phytohemagglutinin-M (Gibco). Cytochalasin-B (Sigma) at a final concentration of 3 μg/ml was added 44 h after the establishment of the culture. After an incubation period of 72 h at 37°C, cultures were treated with 0.85% NaCl and fixative

Table 1
Frequency of cells with micronuclei induced by different gamma-radiation doses on binucleated lymphocytes of 4 healthy young individuals (group A, individuals 1–4)

Dose (cGy)	Number of cells scored	Cells with zero MN (y_{i0})					Cells with one MN (y_{i1})					Cells with two or more MN (y_{i2})					number of cells with MN(%)	number of MN (MN/cell)
		1	2	3	4	Total	1	2	3	4	Total	1	2	3	4	Total		
0	2373	610	774	453	504	2341	19	4	4	4	31	1	0	0	0	1	32 (1.35)	33 (0.014)
20	2662	433	579	579	1020	2611	7	11	15	12	45	0	2	4	0	6	51 (1.91)	61 (0.023)
50	1991	432	460	460	497	1849	33	31	32	21	117	1	9	13	2	25	142 (7.13)	173 (0.087)
100	2047	401	484	440	486	1811	44	40	58	47	189	16	15	7	9	47	236 (11.53)	300 (0.146)
200	1611	632	781	316	475	2204	104	110	95	16	325	14	16	40	12	82	407 (15.59)	510 (0.195)
300	2442	298	427	572	437	1734	97	129	201	74	501	45	55	95	12	207	708 (29.00)	984 (0.403)
400	2398	352	335	606	328	1621	125	116	152	130	523	35	48	123	48	254	777 (32.40)	1102 (0.459)
500	1746	178	195	207	425	1005	112	144	109	91	456	66	111	77	31	285	741 (42.44)	1143 (0.655)

(methanol : acetic acid 3:1) in equal volumes and centrifuged for 10 min at 1500 rpm. The supernatant was changed twice for fixative only.

Cell suspensions were dropped on dry slides, fixed at 65°C for 3 min and stained with 5% Giemsa in phosphate buffer, pH 7.0.

2.4. Cytogenetic analysis

The frequencies of spontaneous and induced MN were analyzed in a total of about 500 binucleated cells for each donor at each radiation dosis. MN were observed at a magnifying power of 320 × and identified according to the criteria of Countryman and Heddle (1976) and Fuhrmann et al. (1992): they were scored only in cells with preserved cytoplasm, non-confluent, at the same focal plan, with diameters up to one-third of the main nucleus, having the same or a lighter color. Cells with more than 5 MN were not considered, to avoid the possible inclusion of lymphocytes in the process of nuclear fragmentation.

2.5. Criteria for analysis

The cellular radiosensitivity of the individuals under study was evaluated through the analysis of the number of MN per cell and the frequency of lymphocytes with zero, one and two or more MN.

2.6. Statistical analysis

The radiation doses used in the experiment and the frequencies of cells with zero, one and two or more micronuclei are used to obtain a functional relationship between dose and response. The dose–response model presented here uses a normal approximation for a transformation of the raw frequency (the log-odds) with unlimited domain (for details, see Madruga et al., 1994).

The purpose of the statistical analysis is to present the dose–response model that better adjusts to our data. Note that for each dose i we observe a vector $y_i = (y_{i0}, y_{i1}, y_{i2})$ of frequency. y_{i0} is the number of cells with no micronucleus, y_{i1} is the number of cells with one micronucleus and y_{i2} is the number of cells with two or more micronuclei. The correspondent parameter of interest, population proportion, is π_{i0}, π_{i1} and π_{i2}, respectively. We estimate these proportions as described in Madruga et al. (1994). We have also compared the 3 groups in relation to the number of cells with micronuclei. We have used a χ^2 for homogeneity.

3. Results

Results from cytogenetic analyses are shown on Tables 1–3, where the frequencies of cells with zero, one, and two or more MN and radiation doses are presented for all individuals under study.

3.1. Spontaneous frequencies

The analysis of the frequencies of cells with spontaneous MN (0 cGy) in lymphocytes showed that individuals in group C (with cancer) have similar values to those in group A (healthy young), but lower than group B (healthy older) (Table 4). Groups

Table 2
Frequency of cells with micronuclei induced by different gamma-radiation doses on binucleated lymphocytes of two healthy older individuals (group B, individuals 5 and 6)

Dose (cGy)	Number of cells scored	Cells with zero MN (y_{i0})			Cells with one MN (y_{i1})			Cells with two or more MN (y_{i2})			Number of cells with MN(%)	Number of MN (MN/cell)
		5	6	Total	5	6	Total	5	6	Total		
0	953	426	494	920	19	12	31	1	1	2	33 (3.46)	37 (0.039)
20	1038	501	488	989	24	17	41	6	2	8	49 (4.72)	59 (0.057)
50	1003	478	455	933	19	37	56	4	10	14	70 (6.98)	86 (0.086)
100	1085	444	495	939	50	64	114	12	20	32	146 (13.46)	198 (0.182)
200	1037	425	369	794	76	100	176	28	39	67	243 (23.43)	317 (0.306)
300	951	369	314	683	116	93	209	35	24	59	268 (28.18)	337 (0.345)
400	1105	365	377	742	142	114	256	64	43	107	363 (32.85)	494 (0.447)
500	1241	428	343	771	210	117	327	95	48	143	470 (37.87)	665 (0.536)

Table 3
Frequency of cells with micronuclei induced by different gamma-radiation doses on binucleated lymphocytes of 3 basocellular carcinoma patients (group C, individuals 7–9)

Dose (cGy)	Number of cells scored	Cells with zero MN (y_{i0})				Cells with one MN (y_{i1})				Cells with two or more MN (y_{i2})				Number of cells with MN (%)	Number of MN (MN/cell)
		7	8	9	Total	7	8	9	Total	7	8	9	Total		
0	1511	498	492	502	1492	6	5	4	15	1	1	2	4	19 (1.26)	27 (0.018)
20	1527	508	509	461	1478	13	11	16	40	3	2	4	9	49 (3.21)	54 (0.035)
50	1689	501	492	511	1504	53	59	58	170	4	5	6	15	185 (10.95)	222 (0.131)
100	1443	430	440	435	1305	43	47	39	129	2	4	3	9	138 (9.56)	153 (0.106)
200	1472	411	408	412	1231	77	68	84	229	3	4	5	12	241 (16.37)	280 (0.190)
300	1553	384	393	379	1156	100	98	103	301	31	30	35	96	397 (25.56)	549 (0.353)
400	1469	340	354	344	1038	118	115	116	349	26	28	28	82	431 (29.34)	614 (0.418)
500	1615	332	343	326	1001	178	175	177	530	27	29	28	84	614 (38.01)	960 (0.594)

Table 4
Spontaneous frequency of MN in the 3 groups studied

Donors	Spontaneous frequency			
	MN/cell	% of cells with one MN	% of cells with two or MN	% of micronucleated cells
Healthy young group (A)	0.014	1.306	0.042	1.35
Healthy older group (B)	0.039	3.253	0.210	3.46
Cancer patients group (C)	0.018	0.993	0.265	1.26

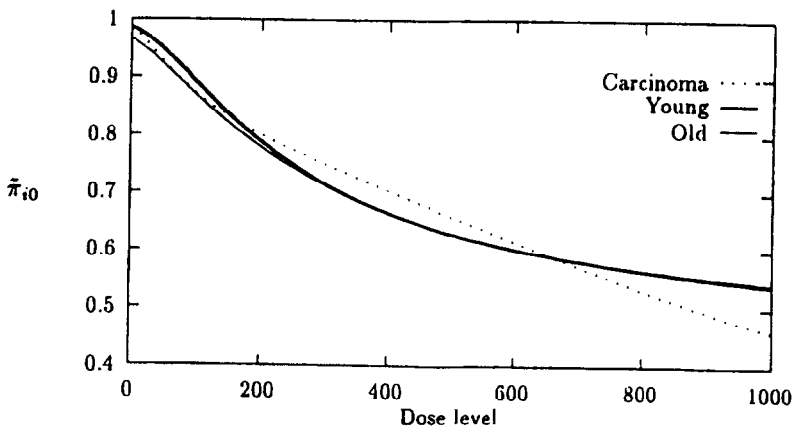

Fig. 1. Proportion of binucleated cells with zero MN adjusted for each group.

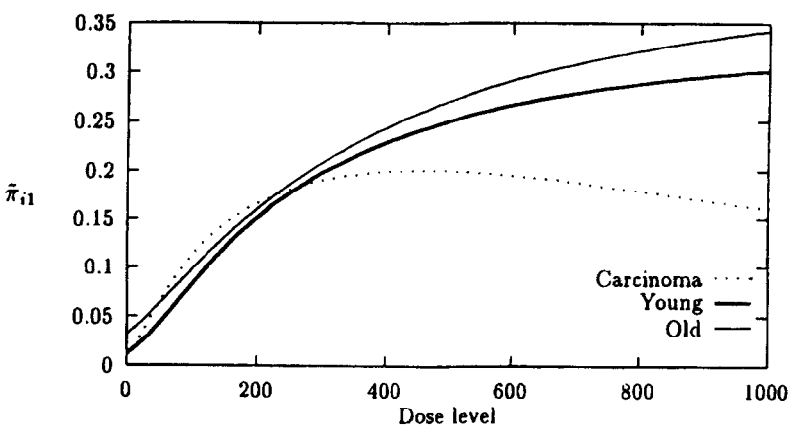

Fig. 2. Proportion of binucleated cells with one MN adjusted for each group.

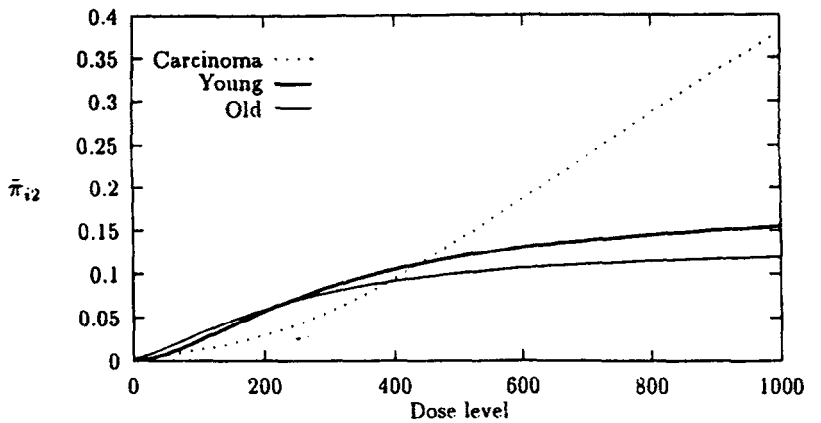

Fig. 3. Proportion of binucleated cells with two or more MN adjusted for each group.

A and C were not significantly different ($\chi_1^2 = 0.059$); comparing group B with the other two together, a highly significant difference was found ($\chi_1^2 = 19.21$).

3.2. Radioinduced frequencies

Both cancer patients and healthy subjects showed an increase in the frequency of lymphocytes with MN as well as in the number of MN per cell, as radiation doses increase (Tables 1–3).

The adjusted curve (Fig. 1) shows that, both in cancer patients and healthy subjects, the proportion of cells not affected by radiation, with no MN, was not different. However, Fig. 2 and Fig. 3 show that the carcinoma group (C) differ from the other two (groups A and B) which are similar, in relation to the distribution of MN. Group C showed a decrease in the proportion of cells with one MN as radiation dose increased, while healthy groups (A and B) showed a rising non-linear curve (Fig. 2). When lymphocytes with 2 or more MN were considered, group C showed a pronounced slope curve from 400 cGy (Fig. 3); healthy groups showed a more stable response at the same dose range. The construction of Figs. 1–3 is presented in Madruga et al. (1996).

4. Discussion

Results from the cytogenetic analysis of the 3 groups of donors (A, B and C) showed that the two older healthy subjects (B, aged 46 and 50 years) had the highest spontaneous frequencies of cells with MN; patients with cancer (C, aged 68, 67 and 47 years) did not differ from healthy young subjects (A, aged 20–27 years).

Hall and Wells (1988), Huber et al. (1989), Migliore et al. (1991) and Hando et al. (1994) found an increase in the number of cells with spontaneous MN with increasing age.

It is accepted that DNA repair mechanisms are responsible, at least partially, for the maintenance of DNA integrity, after spontaneous or induced DNA damage (Fenech and Morley, 1986; Hsu et al., 1989). According to this assumption, an increase with age in the spontaneous frequency of MN may be indicative of an increasing vulnerability of the genome with age, in vivo. It is possible, then, that the higher frequencies of spontaneous MN in groups B and C result from an increasing deficiency in DNA repair with age.

The increase of spontaneous MN with age may be associated to several factors, such as induction of aneuploidy and/or increased rates of spontaneous chromosome breaks (Fenech and Morley, 1986).

Several other authors (de Jong et al., 1988; Prosser et al., 1988; Gantemberg et al., 1991), however, have not found a significant difference between young and older subjects and attributed their findings to an interindividual difference or to the small age range (27–43 years) in the case of Gantemberg et al. (1991).

It is difficult to explain the fact that the group of healthy older subjects (group B) had higher spontaneous frequencies of cells with MN than the cancer patients group: the multiplicity of exogenous and endogenous factors interacting in the mutagenic response might be responsible. Interindividual variability may result from immune, hormonal, vascular, metabolic and nutritional factors, as well as from genetic constitution, life style, etc. Another factor that must be taken into account is the small number of subjects studied. Besides, the frequency of spontaneous chromosome breaks is usually low in individuals, including those with cancer. Individuals with xeroderma pigmentosum do not have a high frequency of spontaneous chromosome breaks; only when exposed to UV light do they express chromosome fragility. For all these reasons, the analysis of sensitivity to a mutagen may give better information to evaluate the genetic instability than the spontaneous rate of chromosome aberrations or MN (Hsu, 1987).

The proportions of radiation-damaged lymphocytes were similar in the 3 groups: patients with cancer (C) and healthy subjects (A and B), with a close related increase in the frequency of micronucleated cells with increasing radiation doses. Similar results were obtained by other authors (Lloyd et al., 1986; Balasem et al., 1993) who also found an increase in the frequency of MN related to the radiation doses.

The magnitude of the induced lesions, however, was different, as demonstrated by the analysis of the distribution of radioinduced MN in binucleated lymphocytes: in the cancer group, the proportion of less damaged cells (with only one MN) decreased as radiation dose increased, giving place to more damaged cells (with several MN) in the highest doses (> 400 cGy). To better visualize these phenomena we expanded the dose range to 1000 Gy. Note that we are only drawing the adjusted theoretical model and not the actual data. In this aspect, we might conclude that group C was more sensitive to radiation than the healthy groups.

Although the older subjects (B) showed a higher frequency of spontaneous MN, sensitivity to ionizing radiation does not seem to be associated with age, since their response was similar to that of young subjects (A). According to Hsu (1987), spontaneous chromosome instability and sensitivity to mutagens are independent phenomena.

Darroudi et al. (1995) observed a higher radiosensitivity in patients with lymphoma and aplastic anemia (congenital or acquired disorder) in comparison to normal subjects, as shown by higher frequencies of chromosomal abnormalities in X-irradiated lymphocytes at 0.5 and 1 Gy. They also observed no differences in spontaneous chromosome damage between patients and normal controls. They propose that these patients may differ in their capacity for repairing ionizing radiation damage to DNA strands, not excluding, however, the existence of other types of DNA lesions involved in the production of chromosome aberrations.

In our study, the carcinoma patients (who are sensitive to UV light) are probably deficient in excision repair, the mechanism activated for damage induced by UV light, the main carcinogen involved in skin cancer (Brash et al., 1991; Kamb, 1994). Individuals may be sensitive to only one agent, several agents or none (Hsu, 1987).

Considering that the main lesion caused by ionizing radiation is the DNA double-strand break (IAEA, 1986) and that MN originate predominantly from acentric fragments, the results allow us to assume that the patients with basocellular carcinoma in this study may be less efficient than the healthy subjects in repairing DNA double-strand breaks. It is known that individuals with xeroderma pigmentosum, ataxia telangiectasia and Fanconi's anemia represent the extreme end of the distribution spectrum of mutagen susceptibility (Setlow, 1978). The overwhelming majority of individuals in the human population should not have such severely defective DNA repair mechanisms. However, a degree of mutagen susceptibility may exist in some individuals, because of mild imperfections of the DNA repair systems. Conceivably, such defects may cover a wide range, from slightly inefficient to moderately defective. If so, individuals with mild degrees of mutagen sensitivity may be more likely to accumulate mutations and chromosome damage in their cells when they are exposed to environmental mutagens (Hsu, 1987).

As cells carrying many DNA lesions survive and increase in number, favorable conditions for neoplastic transformation may accumulate, making the individual more susceptible to cancer. The fact that

cancer patients show a significantly higher frequency of bleomycin-induced chromatid breaks (Hsu et al., 1989) seems to give support to this assumption.

The results indicate that not only the frequency of cells with MN, but also the number and distribution of MN, can be used as a valuable parameter to estimate both genetic instability and response to environmental mutagens.

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