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Evaluation of the effect of ^{90}Sr β-radiation on human blood cells by chromosome aberration and single cell gel electrophoresis (comet assay) analysis

Elaine Mendes de Oliveira, Miriam Fussae Suzuki, Patrícia Alves do Nascimento, Márcia Augusta da Silva, Kayo Okazaki*

Instituto de Pesquisas Energéticas e Nucleares (IPEN), Comissão Nacional de Energia Nuclear (CNEN/SP), Supervisão de Radiobiologia (TBR), Travessa R, 400, Caixa postal 11049, Cidade Universitária, Pinheiros, 05508-900 Sao Paulo, SP, Brazil

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Abstract

Among various environmental genotoxins, ionizing radiation has received special attention because of its mutagenic, carcinogenic and teratogenic potential. In this context and considering the scarcity of literature data, the objective of the present study was to evaluate the effect of ^{90}Sr β-radiation on human cells. Blood cells from five healthy donors were irradiated in vitro with doses of 0.2–5.0 Gy from a ^{90}Sr source (0.2 Gy/min) and processed for chromosome aberration analysis and for comet assay. The cytogenetic results showed that the most frequently found aberration types were acentric fragments, double minutes and dicentrics. The α and β coefficients of the linear–quadratic model, that best fitted the data obtained, showed that ^{90}Sr β-radiation was less efficient in inducing chromosome aberrations than other types of low linear energy transfer (LET) radiation such as ^{3}H β-particles, ^{60}Co γ-rays, ^{137}Cs and ^{192}Ir and X-rays. Apparently, ^{90}Sr β-radiation in the dose range investigated had no effect on the modal chromosome number of irradiated cells or on cell cycle kinetics. Concerning the comet assay, there was an increase in DNA migration as a function of radiation dose as evaluated by an image analysis system (tail moment) or by visual classification (DNA damage). The dose–response relation adequately fitted the non-linear regression model. In contrast to the cytogenetic data, ^{90}Sr β-radiation induced more DNA damage than ^{60}Co γ-radiation when the material was analyzed immediately after exposures. A possible influence of selective death of cells damaged by radiation was suggested.

Keywords: Microgel electrophoresis; Chromosome aberration; ^{90}Sr β-radiation; Human blood cells; DNA damage and repair; Dose–response curve

1. Introduction

Studies concerning the effects of different types of ionizing radiation on human cells are very important not only from a radiobiological viewpoint but also for dosimetric and therapeutic purposes. Although different types of ionizing radiation have similar qualitative effects on biological systems, the type of radiation may induce a significant quantitative difference in cellular response, even when the total amount of energy deposited is the same [1]. This occurs because biological effects are closely related to linear energy transfer (LET), i.e. mean quantity of energy deposited in the system per distance unit (keV/μm).

* Corresponding author. Tel.: +55-11-3816-9236; fax: +55-11-3816-9232.
E-mail address: kokazaki@net.ipen.br (K. Okazaki).

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In general, high LET radiation (α-particles and fission fragments) is more efficient in inducing biological damage than low LET radiation (γ- and X-rays, β-particles) because all the energy is deposited within a short distance, causing dense ionization in the trajectory [2].

Despite the considerable quantity of published work about the cytogenetic effects of γ-radiation [3,4], X-rays [5], α-particles [6,7] and neutrons [8,9], little information is available about the effects of β-particles on human cells.

The importance of analyzing the effects of β-radiation at the cellular level is its implication in diverse areas, such as nuclear medicine [10], nuclear industries [11,12] and biomedicine [13], increasing the risk of occupational and accidental exposure. The use of some β-emitter radionuclides, such as ^{32}P, ^{186}Re, ^{89}Sr, ^{131}I and ^{153}Sm in nuclear medicine is due to their low tissue penetration, with reduced toxicity to neighboring tissues [14]. ^{90}Sr in plates has been used for the treatment of superficial cutaneous lesions reaching a few millimeter in depth [13].

In this context, the study of ^{90}Sr as a pure source of high energy β-particles is of particular interest. Its physical half-life is 28 years and it decays by β-emission ($E = 0.54$ MeV), turning into ^{90}Y ($E = 2.27$ MeV), which also decays by β-emission [15]. As ^{90}Sr is a fission product of ^{235}U, it may contaminate the atmosphere by escaping during or after nuclear fuel reprocessing and enter the terrestrial food chain, affecting population health. The high energy of β-particles of the Sr/Y system and the ^{90}Sr deposited in the organism by ingestion or inhalation may lead to chronic bone and red marrow irradiation and consequently affect blood cells [13].

Information about the potential of ^{90}Sr for inducing bone tumor, leukaemia and genetic effects has been described by UNSCEAR [15], but its cytogenetic effects have been little explored.

The cytogenetic effect of β-radiation on human cells reported in the literature refers to the exposure of blood lymphocytes to ^{3}H [11,12,16] and ^{131}I [17–19]. The effects of ^{90}Sr have been investigated by Hall and Wells [20] and Mill et al. [21] using the micronucleus assay as a parameter for cytogenetic damage evaluation and by Vulpis and Scarpa [22] using induced chromosome aberration analysis, with the radiation source being in direct contact with the blood sample. The association between the presence of chromosome aberrations in bone marrow and the induction of dermatitis and malignant diseases in workers who manipulate luminescent paint containing ^{90}Sr and ^{226}Ra has been reported by Müller et al. [23].

In the present study, we evaluated the qualitative and quantitative effects of ^{90}Sr β-radiation on human peripheral lymphocytes irradiated in vitro by chromosome aberration analysis and by single cell gel electrophoresis (comet assay). The chromosome aberration technique detects unrepaired DNA lesions, particularly double strand breaks, while the comet assay detects the lesions occurring in individual cells immediately after exposure, including DNA single and double strand breaks and alkali label sites. All these lesions are induced by ionizing radiation and represent important damage related to cell death, ageing, malformation and cancer.

2. Materials and methods

2.1. Donors

Blood samples were obtained from five healthy female donors, 23–41 years, non-smokers with no history of radiotherapy, no alcohol or medicine consumption and no disease at the time the blood samples were analyzed.

This project was evaluated and approved by the Ethics in Research Committee of the Institute for Nuclear and Energy Research (IPEN) — CNEN/SP (no. 006/CEP). All donors were submitted to a questionnaire to obtain information related to their life style, such as dietary habits, medical history and exposure to chemical and physical agents.

2.2. Blood sample collection

About 10 ml of blood was collected by venipuncture from each donor into heparinized syringes. All samples were analyzed by two methods, i.e. chromosome aberration analysis and single cell gel electrophoresis (comet assay).

2.3. Irradiation conditions

Blood samples were fractionated and irradiated with a ^{90}Sr source ($E_{max} = 2.27$ MeV, $E_{avg} = 1.13$ MeV)

(Amersham Buchler), at a dose rate of 0.2 Gy/min, with doses of 0.2, 0.5, 1.0, 3.0, and 5.0 Gy, at room temperature and in the presence of molecular oxygen. One fraction of each sample was used as control (0 Gy).

The irradiation system for sterile condition and physical dosimetry was supervized by the Departamento de Metrologia das Radiações from IPEN — CNEN/SP. The source was calibrated using an extrapolation chamber 23391 (PTW-Freiburg). The apparatus was made of acrylic, since this material has a density approximately equal to that of water and similar to that of biological tissues and its low atomic number prevents X-ray emission when the material is irradiated with β-particles. The short penetration trajectory of ^{90}Sr was also considered since the average energy particle penetrates soft tissues to a depth of only 10 mm [15].

Blood samples were fractionated and placed in nylon vials, 0.7 cm high and 2.5 cm in diameter, forming a 1 mm blood layer for irradiation.

2.4. Chromosome aberration technique

For chromosome damage observation, a culture with 0.5 ml of whole blood from each donor, 4.5 ml of MEM medium, 10% of foetal calf serum (Cultilab, Campinas, Brazil), 100 μl of phytohaemagglutinin (5 μg/ml Gibco BRL) and 60 μl of BrdU (Sigma) was incubated at 37°C for 48 h. Nearly 2 h before fixation, 30 μl of Colcemid (0.5 μg/ml) (Sigma) was added. At the end of incubation, cells were harvested by centrifugation, submitted to hypotonic treatment with 0.075 M potassium chloride and 1% sodium citrate (Merck) (3:1) and then fixed in a fresh fixative solution of methanol and acetic acid (Merck) (3:1). This cell suspension was transferred to microscope slides, in a pre-heated humid atmosphere at 65°C, then air-dried overnight at room temperature. The slides were stained with Hoescht 33258 (Sigma), covered with 0.5 ml of McIlvaine buffer, pH 8.0, exposed to UV light (245 nm) for 40 min at 60°C, rinsed with distilled water, air-dried, and stained for 10 min with 5% Giemsa (Sigma) in Sorensen phosphate buffer, pH 6.8.

2.4.1. Criteria for analysis

For each dose, 100–360 metaphases per donor were analyzed. Cells with $2n$, $2n-1$ and $2n-2$ chromosomes were considered. Different kinds of structural aberrations were identified using AIEA criteria [24]. Each rearrangement aberration was considered with only one acentric fragment, while the other fragments, if any, were considered separately. All terminal and interstitial deletions, i.e. acentric fragments and double minutes, were classified as acentric aberrations. The metaphases were classified according to three basic criteria: presence of structural aberrations, number of chromosomes and first or second mitotic division.

2.5. Single cell gel electrophoresis (comet assay)

The alkaline version of the comet assay described by Singh et al. [25] was adopted, with some modifications.

For evaluation of DNA damage, all samples were maintained in ice immediately after the irradiations for the evaluation of initial damage. For each blood sample, irradiated or not, microscope slides (in duplicate) were covered with 1.5% normal melting agarose (Sigma) dissolved in PBS (Ca^{2+} and Mg^{2+} free) and maintained overnight at room temperature. A volume of 5 μl of blood dissolved in 90 μl of 0.5% low melting agarose (Sigma, PBS Ca^{2+} and Mg^{2+} free) was placed on this gel layer at 37°C. After solidification at 4°C (5–10 min), the slides were placed vertically in a cuvette with lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% sodium sarcosinate, 1% Triton X-100 and 10% DMSO, pH 10.0) for 2 h at 4°C to remove proteins. After lysis, the slides were placed side by side in a horizontal electrophoresis tank (17 cm × 20 cm) (Permatron) and immersed in alkaline buffer, pH 13.0 (1 mM EDTA and 300 mM NaOH) for 30 min to allow DNA damage expression and submitted to electrophoresis (25 V, 300 mA) (Pharmacia) for 30 min at 4°C. The slides were then neutralized with 0.4 M Tris buffer, pH 7.5, fixed with absolute ethanol for 10 min [26], and stained with 40 μl ethidium bromide (20 μg/ml) (Sigma). The comets were analyzed under a fluorescence microscope (Carl Zeiss) at 400×, with an exciting filter of 515–560 nm and a barrier filter of 590 nm and photographed on black and white Kodak T-Max ASA 400 film.

2.5.1. Criteria of analysis

For each radiation dose, 52 comets were randomly analyzed per donor. Two methods were used for

quantitative evaluation of damage: the image analysis system Casys (Synoptics) and visual classification [27]. For the image analysis system, tail moment was used as it is considered to be the most sensitive parameter of DNA damage, defined as the product of total fluorescence intensity by the distance between the tail mass center and the head mass center, as described by the program developer.

The extent of DNA migration was evaluated by visual analysis according to the criteria established by Jaloszynski et al. [27]. The comets were classified into five categories (0–4) according to DNA damage. Comets with a bright head and no tail were classified as class 0 and comets with a small head and long diffuse tails, as class 4, i.e. highly damaged cells. Comets with intermediate characteristics were classified as classes 1, 2 or 3.

Quantitative DNA damage (DD) was estimated by the formula described by Jaloszynski et al. [27]:

$$\mathrm{DD} = \frac{(n_1 + 2n_2 + 3n_3 + 4n_4)}{\Sigma/100}$$

where DD is the DNA damage in arbitrary units (au), n_1–n_4 the number of classes 1–4 comets, and Σ is the total number of scored comets, including class 0.

2.6. Statistical analysis

The dose–response curves obtained for dicentric and acentric aberrations were fitted to linear ($Y = \alpha D$), quadratic ($Y = \beta D^2$) and linear–quadratic models ($Y = \alpha D + \beta D^2$), where Y is the number of aberrations/cell, α the linear coefficient, β the quadratic coefficient, and D is the dose (Gy).

The dose–response curves for DNA migration were fitted by non-linear regression (one phase exponential decay)

$$Y = A\,\mathrm{e}^{(-kD)} + B$$

where Y is the tail moment or DNA damage (au), A, B, k the constants, and D is the radiation dose (Gy).

The statistical analyses were done using the Graph Pad Prism software.

3. Results

3.1. Damage evaluation by chromosome aberration analysis

The cytogenetic analysis of peripheral lymphocytes irradiated in vitro with ^{90}Sr showed various types of structural chromosome aberrations which increased as a function of the radiation dose (Table 1). The most frequent types were acentric fragments, double minutes and dicentrics. No acentric ring was observed.

The dose–response relationships for dicentric and acentric aberrations (acentric fragments and double minutes) are illustrated in Fig. 1. The α and β coefficients of the models used to fit the curves with their respective standard error (S.E.) are presented in Table 2. Values of r^2 showed that the data better fitted the linear–quadratic model for both dicentric and acentric aberrations.

The α and β coefficients for the induction of dicentric and acentric aberrations (double minutes, acentric fragments and acentric rings) at low LET radiation

Table 1
Frequency of different types of chromosome aberration found in lymphocytes irradiated in vitro with ^{90}Sr[a]

Dose (Gy)	Number of cells scored	DC	CR	AA	CG	Total number of aberrations	Number of dicentric/cell	Number of acentric/cell	Number of aberration/cell
0.0	1546	2	0	8	3	13	0.0013	0.005	0.008
0.2	1283	1	0	14	4	19	0.0008	0.011	0.015
0.5	1356	6	1	50	4	61	0.0044	0.037	0.045
1.0	1097	11	0	54	3	68	0.0100	0.049	0.062
3.0	863	52	3	249	16	320	0.0600	0.288	0.371
5.0	486	75	7	344	4	430	0.1540	0.708	0.885

[a] Where DC is the dicentric, CR the centric ring, AA the acentric aberration (acentric fragment + double minutes), and CG is the chromatidic and chromosomic gaps.

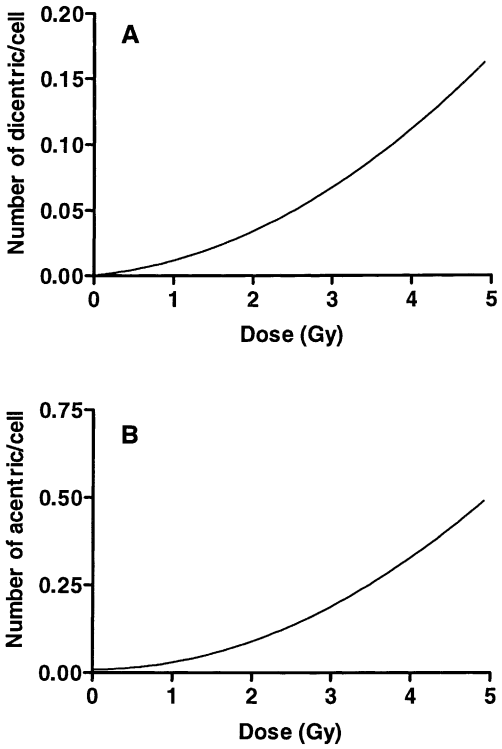

Fig. 1. Dose–response curves adjusted by linear–quadratic model for induction of dicentrics (A); and acentric aberrations (B), obtained from peripheral lymphocytes irradiated with ^{90}Sr.

reported in the literature are presented in Tables 3 and 4, respectively, for comparison.

Table 5 shows the frequencies of metaphases with a modal ($2n = 46$) and hypomodal ($2n-1$ and $2n-2$) chromosome number observed in lymphocytes irradiated in vitro. The frequency of cells presenting 46 chromosomes was higher than 97% at all doses analyzed. Similarly, the frequency of cells in second division was less than 6.5%.

3.2. Damage evaluation by single cell gel electrophoresis

Blood cells irradiated with ^{90}Sr and analyzed by the SCGE assay presented a bright comet head and a tail whose fluorescence and length increased with radiation dose (Fig. 2).

The data obtained for blood cells from five donors by using two parameters, tail moment (au) as analyzed by Casys (Synoptics) and DNA damage (DD) by visual analysis, are summarized in Table 6. There was an increase in DNA migration with increasing dose, evaluated either with the image analysis system (tail moment) or by visual classification (DD).

The dose–response curves generated for DNA migration using a non-linear regression model with two parameters are shown in Fig. 3. The data obtained for DNA damage after exposure to ^{90}Sr by visual classification were compared with the data obtained with ^{60}Co in peripheral lymphocytes processed immediately after irradiation [28] in our laboratory. The estimated parameters of the model and the coefficients of the fitted model (r^2) for tail moment and DD are listed in Table 7. The coefficients of the non-linear regression model (r^2>0.90) showed that the data adequately fitted the model.

Fig. 4 presents the histograms of the distribution of cells with various classes of DNA damage (0–4) induced by ^{90}Sr β-radiation and ^{60}Co γ-radiation just after exposure. The values of cells with various categories of damage for each radiation dose, β and γ, are listed in Table 8. It can be seen that cells without any apparent damage (class 0) predominated in non-irradiated blood samples, with the occurrence of a small population of class 1 cells, i.e. cells with low damage, in both cases (*A* and *B*). In irradiated

Table 2
Values of parameters from dose–response relation for induction of chromosome aberrations with β-radiation of ^{90}Sr

Type of chromosome aberration	Model	α (±S.E.) × 10^{-2} Gy^{-1}	β (±S.E.) × 10^{-2} Gy^{-2}	α/β (Gy)	r^2
Dicentric	Linear	3.213 ± 0.435	–	–	0.67
	Quadratic	–	0.662 ± 0.084	–	0.70
	Linear–quadratic	0.580 ± 1.668	0.549 ± 0.336	1.05	0.70
Acentric	Linear	13.880 ± 0.835	–	–	0.91
	Quadratic	–	2.850 ± 0.134	–	0.94
	Linear–quadratic	3.289 ± 2.587	2.208 ± 0.522	1.49	0.95

Table 3
Values of α and β coefficients of linear–quadratic model with respective standard errors (S.E.) for the induction of dicentrics in human peripheral lymphocytes for low LET radiation

Type of radiation	Dose rate (Gy/min)	Dose range (Gy)	α (±S.E.) $\times 10^{-2}$	β (±S.E.) $\times 10^{-2}$	Reference
^{60}Co γ-rays	0.003	0.25–8.00	1.76 ± 0.76	2.91 ± 0.47	[3]
^{60}Co γ-rays	0.500	0.25–8.00	1.57 ± 0.29	5.00 ± 0.20	[3]
^{60}Co γ-rays	0.100	0.50–7.00	3.37 ± 1.63	5.23 ± 0.55	[34]
^{60}Co γ-rays	0.017	–	0.90 ± 4.00	4.17 ± 0.28	[29]
^{192}Ir γ-rays	0.120	0.50–4.00	3.18 ± 1.80	6.09 ± 0.72	[36]
^{192}Ir γ-rays	–	–	3.20	6.10	[35]
^{137}Cs γ-rays	0.07–0.44	–	6.80	0.68	[32]
^{137}Cs γ-rays	0.490	–	4.33 ± 1.50	4.31 ± 0.42	[33]
X-rays	0.500	–	4.10 ± 0.50	2.60 ± 0.40	[37]
X-rays	0.350	0.05–7.00	1.80 ± 0.48	5.48 + 0.46	[11]
X-rays	0.500	–	4.04 ± 0.30	5.98 ± 0.17	[29]
HTO β-rays	0.020	0.10–2.00	15.60	11.40	[12]
HTO β-rays	0.01–0.05	0.25–7.00	12.80 ± 1.38	5.78 ± 0.61	[11]
^{90}Sr β-rays	0.130	0.138–2.76	8.22 ± 1.72	8.81 ± 1.14	[22]
^{90}Sr β-rays	0.200	0.20–5.00	0.58 ± 1.67	0.55 ± 0.34	Present study

Table 4
Values of α and β coefficients of linear–quadratic model with respective standard errors (S.E.) for the induction of acentric aberration (acentric fragments + double minutes + acentric rings) found in peripheral lymphocytes after low LET irradiation in vitro

Type of radiation	Dose rate (Gy/min)	Dose range (Gy)	α (±S.E.) $\times 10^{-2}$ Gy^{-1}	β (±S.E.) $\times 10^{-2}$ Gy^{-2}	Reference
X-rays	0.160	0.05–0.5	9.90 ± 0.74	0.20 ± 1.86	[39]
X-rays	0.500	0.25–4.0	10.37 ± 0.62	2.02 ± 0.07	[31]
X-rays	–	0.03–3.0	2.99 ± 1.66	0.20 ± 5.45	[40]
X-rays	1.000	0.50–7.0	4.80 + 0.80	4.60 + 0.30	[38]
^{60}Co γ-rays	0.500	0.25–8.0	2.30 ± 0.69	3.90 ± 0.36	[3]
^{60}Co γ-rays	0.003	0.25–8.0	2.64 ± 0.68	1.36 ± 0.27	[3]
^{90}Sr β-rays	0.200	0.20–5.0	3.29 ± 2.58	2.21 ± 0.52	Present study

Table 5
Frequency of metaphases with modal and hypomodal chromosome number in different mitotic cycles after in vitro irradiation of blood samples with various doses of ^{90}Sr

Dose (Gy)	Cells scored	Number of centromeres (%)			Cell cycle (%)	
		46	45	44	1st division	2nd division
0.0	1549	1541 (99.5)	2 (0.1)	6 (0.4)	1508 (97.3)	41 (2.7)
0.2	1283	1267 (98.8)	9 (0.7)	7 (0.5)	1239 (96.6)	44 (3.4)
0.5	1356	1344 (99.2)	6 (0.4)	6 (0.4)	1355 (99.9)	1 (0.1)
1.0	1097	1091 (99.4)	3 (0.3)	3 (0.3)	1029 (93.8)	68 (6.2)
3.0	863	841 (97.4)	12 (1.4)	10 (1.2)	859 (99.5)	4 (0.5)
5.0	486	475 (97.8)	4 (0.8)	7 (1.4)	484 (99.6)	2 (0.4)

samples, less damaged cells were replaced with more damaged cells with increasing radiation dose. The intensity of damage induced by ^{90}Sr at 1 and 3 Gy was similar to that observed after exposure to 2 and 4 Gy ^{60}Co, respectively. At the highest ^{90}Sr dose of 5 Gy there was a predominance of class 4 cells, i.e. extremely damaged cells (81.7%).

It was possible to visualize that ^{90}Sr β-radiation induced more DNA damage than ^{60}Co γ-radiation when the cells were analyzed immediately after exposure.

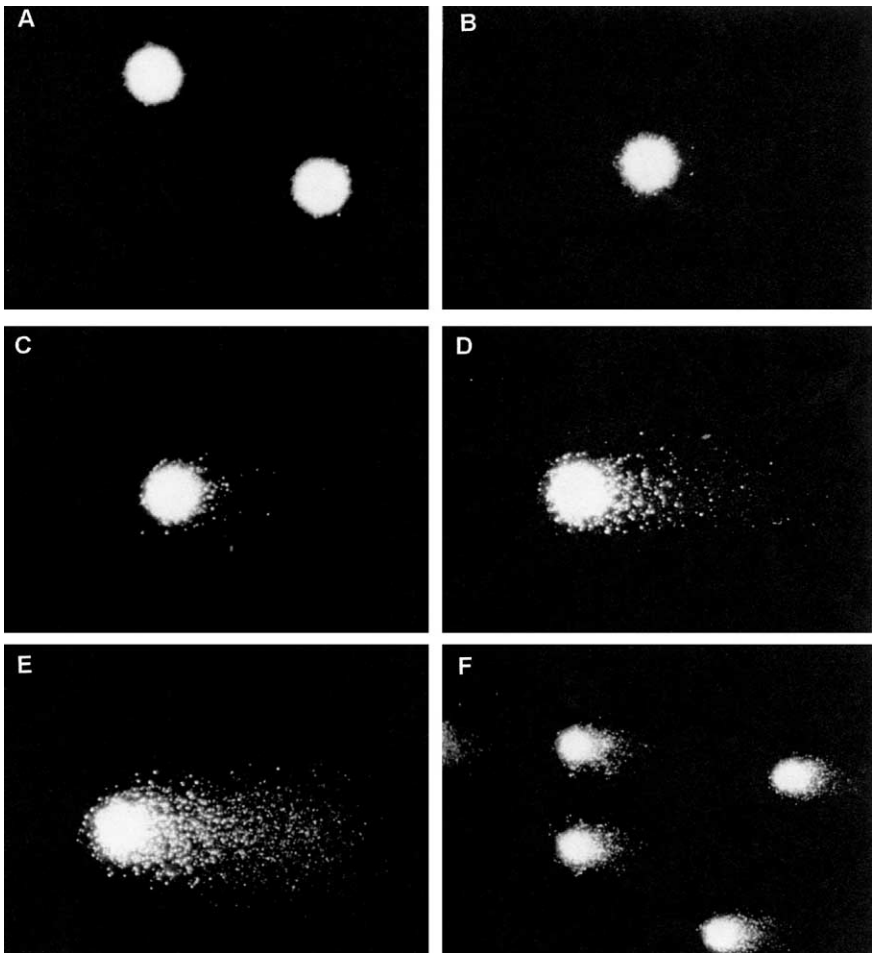

Fig. 2. Photomicrography of blood cells irradiated with ^{90}Sr and processed by comet assay: (A) cells with no DNA migration (class 0); (B) and (C) cells with few DNA migration (classes 1 and 2); (D) very damaged cell (class 3); (E) extremely damaged cell (class 4); (F) aspect of various comets showing long tails.

Table 6
Values of tail moment and DNA damage found in blood samples irradiated with different doses of ^{90}Sr

Dose (Gy)	Scored cells	Tail moment (au)	DNA damage (au)	Cells without DNA migration (%)	Cells with DNA migration (%)
0.0	260	0.011 ± 0.003	8.18 ± 1.82	91.83	8.17
0.2	260	0.019 ± 0.008	96.15 ± 4.96	8.65	91.35
0.5	260	0.025 ± 0.010	174.28 ± 33.15	0.48	99.52
1.0	260	0.041 ± 0.010	232.68 ± 15.95	0.00	100.00
3.0	260	0.101 ± 0.022	320.65 ± 29.36	0.00	100.00
5.0	260	0.126 ± 0.027	381.73 ± 21.81	0.00	100.00

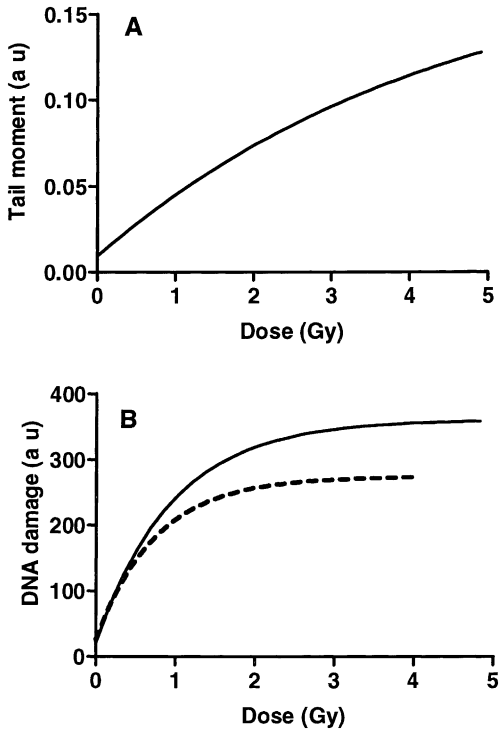

Fig. 3. Dose–response curves adjusted by non-linear regression model for tail moment (A); and DNA damage (B) (——β- and ---- γ-radiation), obtained from blood samples irradiated with ^{90}Sr and processed by comet assay.

4. Discussion

Analysis of the effects of different types of ionizing radiation on biological systems is of great value for a better understanding of their mutagenic and carcinogenic potential in case of accidental or occupational exposure. For this purpose, two techniques were used to evaluate initial and residual lesions (unrepaired or erroneously repaired) in human cells irradiated with ^{90}Sr β-radiation.

The analysis of blood cells irradiated with a β ^{90}Sr source resulted in various types of chromosome aberrations, among them, acentric fragments, double minutes and dicentrics. The same types of structural aberrations were found in cells irradiated with γ-rays [4], α-particles [6], neutrons [9] and X-rays [29], in agreement with the observation that the effects of radiation are qualitatively similar at the chromosomal level.

The quantitative relationship between chromosome aberration and ^{90}Sr β-radiation dose was evaluated using the linear–quadratic model ($Y = \alpha D + \beta D^2$), which showed the best fit.

The biophysical interpretation of this function is that some aberrations originate from only one track (linear component α) that may induce one or two chromosome breaks and others from the integration of two independent tracks (quadratic component β). The αD term corresponds to the lower doses of the dose–response curve, while the βD^2 term stands for the higher doses. Thus, the α/β ratio is equivalent to a radiation dose in which linear and quadratic terms equally contribute to the induction of damage or to the formation of chromosome aberrations [3].

The α/β ratio for dicentric was 1.0 Gy, suggesting that at doses below this value in the dose–response curve dicentrics were predominantly formed by one

Table 7
Estimation of parameters of non-linear regression model utilized to fit dose–response curves for tail moment and DNA damage by comet assay in blood cells irradiated with ^{60}Co and ^{90}Sr

Type of radiation	Parameter of damage evaluation	Coefficients	Estimation	S.E.	r^2
^{90}Sr β-rays	Tail moment	*A*	−0.17	±0.05	0.90
		B	0.18	±0.05	
		k	0.23	±0.11	
^{90}Sr β-rays	DNA damage	*A*	−339.60	±14.80	0.96
		B	360.70	±11.37	
		k	1.05	±0.13	
^{60}Co γ-rays	DNA damage	*A*	−247.10	±17.50	0.93
		B	273.60	±13.68	
		k	1.33	±0.24	

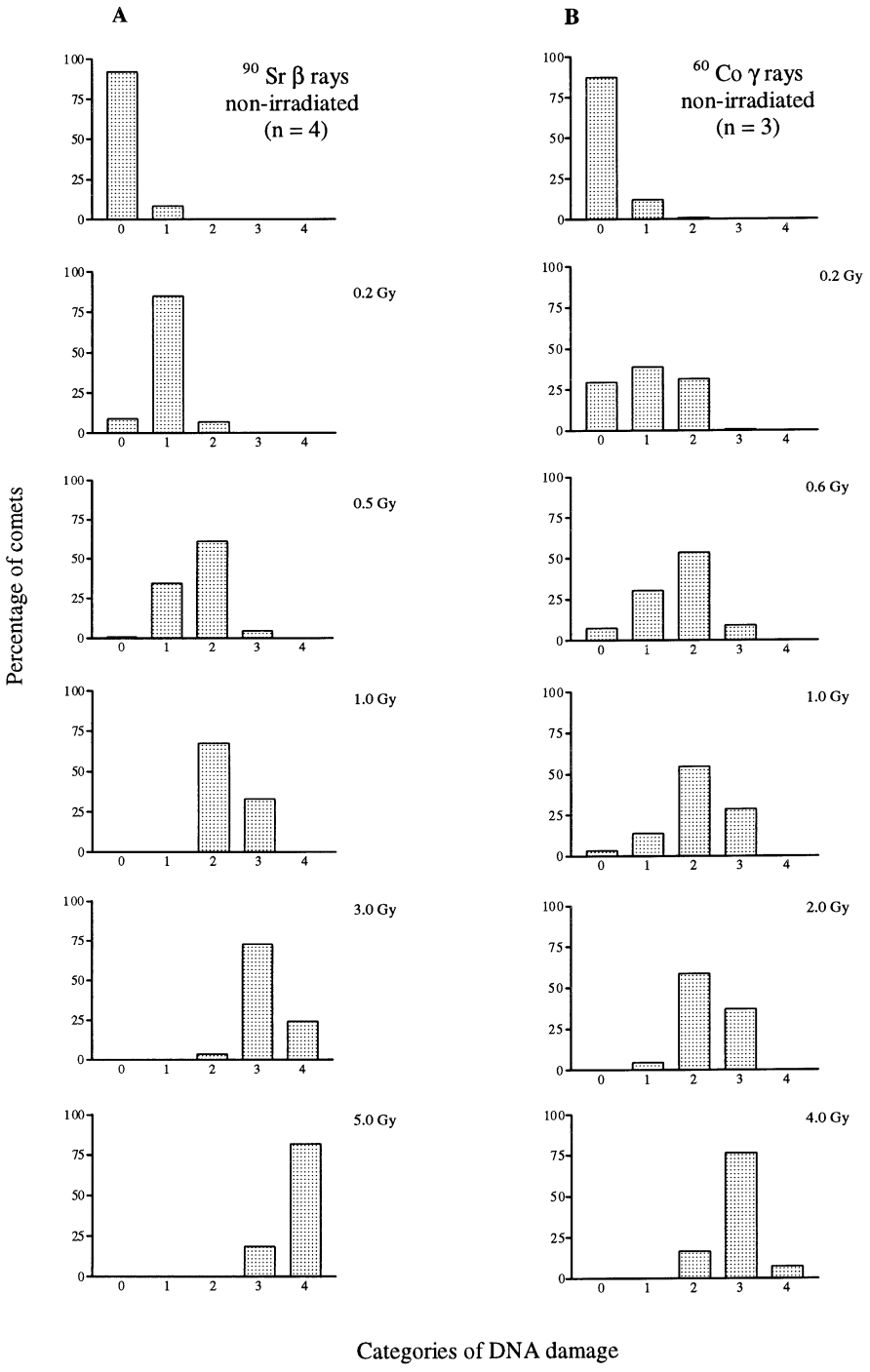

Fig. 4. Frequency distribution of cells with various degrees of DNA damage (class 0–4) for each radiation dose, β of ^{90}Sr (A); and γ of ^{60}Co (B), in blood samples processed by comet assay immediately after the exposures.

Table 8
Frequency of cells in each category of DNA damage by visual analysis of blood cells irradiated with ^{90}Sr β-rays and ^{60}Co γ-rays, processed by comet assay immediately after exposure

Type of radiation	Dose (Gy)	Number of cells	Category of DNA damage (%)				
			0	1	2	3	4
^{90}Sr β-rays	0.0	208	191 (91.8)	17 (8.2)	0	0	0
	0.2	208	18 (8.7)	176 (84.6)	14 (6.7)	0	0
	0.5	208	1 (0.5)	71 (34.1)	127 (61.1)	9 (4.3)	0
	1.0	208	0	0	140 (67.3)	68 (32.7)	0
	3.0	208	0	0	7 (3.4)	151 (72.6)	50 (24.0)
	5.0	208	0	0	0	38 (18.3)	170 (81.7)
^{60}Co γ-rays	0.0	150	131 (87.3)	18 (12.0)	1 (0.6)	0	0
	0.2	154	45 (29.2)	60 (38.9)	48 (31.2)	1 (0.6)	0
	0.6	153	11 (7.2)	46 (30.0)	82 (53.6)	14 (9.1)	0
	1.0	154	5 (3.2)	21 (13.6)	84 (54.5)	44 (28.6)	0
	2.0	154	0	7 (4.5)	90 (58.4)	57 (37.0)	0
	4.0	151	0	0	25 (16.5)	115 (76.1)	11 (7.3)

track only, inducing two breaks, and at doses above it the dicentrics were produced by two independent tracks. The higher α/β ratio for acentric aberrations (1.5 Gy) compared to that for the dicentrics suggests that chromosome aberrations due to one chromosome break as a consequence of one track appear to predominate at higher doses of the dose–response curve.

Actually, the majority of acentric fragments originates from terminal deletions as a consequence of only one chromosome break [30]. In contrast, double minutes result from two breaks occurring on the same arm of one chromosome (interstitial deletion) due to two independent ionizing events. However, there is the possibility that a double minute can result from two close breaks in the same arm caused by one track of electrons, considering the highly compact structure of the chromosome in the eukaryotic cells. While the dicentrics are inter-chromosomal aberrations, acentric fragments and double minutes are intra-chromosomal and probably occur more frequently than the processes involving more than one chromosome. So the frequency of acentric aberrations is higher than that of dicentrics. Besides, in the case of dicentrics, the different distances of interaction between the primary breaks may be responsible for their formation [31].

This information supports the idea that under the conditions of the present study, ^{90}Sr β-radiation was more efficient in inducing lesions by only one ionizing event than by the interaction of two independent ionizing events in the formation of chromosome aberrations.

Comparing the induction of dicentrics by ^{90}Sr β-radiation in the present study with literature data for low LET radiation (Table 3) at similar dose rates and culture conditions, we observed that the α and β coefficients of the linear–quadratic model were lower than those obtained with ^{137}Cs [32,33], ^{60}Co [29,34] and ^{192}Ir [35,36] γ-radiation.

Similarly, comparing the present data with those obtained by others with ^{90}Sr β-radiation [22] and HTO [11,12], we observed that the α and β coefficients of these authors were higher. This can be explained by differences in the irradiation conditions used in [11,12,22], which can influence the results. Vulpis and Scarpa [22] irradiated the blood samples using a ^{90}Sr source in direct contact with the cells. The same condition of exposure to HTO [11,12] probably induced more damage to the cells. In the present study, we used external exposure. Similarly, when the present data are compared with those obtained with X-rays [11,29,37], we can see that X-rays were more efficient in inducing dicentrics than ^{90}Sr when the same irradiation conditions are used, whereas the inverse process is observed when are compared with those of Vulpis and Scarpa [22].

About the induction of acentric aberrations, comparing the present data with those obtained by others with X-rays and ^{60}Co γ-rays (Table 4), it can be seen that

in general, the α coefficients of the linear–quadratic model for X-rays [31,38–40] were higher than that for ^{60}Co γ-rays [3] and ^{90}Sr β-radiation. This may mean that low doses of X-rays are more efficient in inducing acentric aberrations.

Anyway, the interpretation of the dose–response relation for induction of chromosome aberrations by different types of ionizing radiation is difficult because of the complexity of the factors involved. The induction of dicentrics in human lymphocytes obtained in different laboratories has been reported to differ significantly for X-rays, γ-radiation and neutrons. The factors evoked to explain this fact are: donor variability, differences in experimental models, cellular cultures and adopted curve fit models [41].

Therefore, based on the data of Tables 3 and 4, under the conditions of the present study (external exposure), ^{90}Sr β-radiation, in general, was less efficient in inducing chromosome aberrations than ^{3}H β-particles, ^{137}Cs, ^{60}Co, ^{192}Ir γ- and X-rays.

Similar results were obtained by Hall and Wells [20] and by Mill et al. [21], who analyzed the induction of micronuclei in human lymphocytes by ^{90}Sr β-radiation (1.0–1.6 Gy/min) at the dose range of 0.3–3.0 Gy. Hall and Wells [20] and Mill et al. [21] observed that external irradiation with ^{90}Sr was less effective than X-rays (0.5–2.0 Gy/min) in micronucleus induction.

The data obtained showed that there was no difference in number of chromosomes or in frequency of cells in first or second division after ^{90}Sr exposures. Thus, we suggest that ^{90}Sr β-radiation had no influence on modal chromosome number or on the cell cycle within the dose range analyzed.

Concerning SCGE, the comet appearance of cells irradiated with ^{90}Sr was similar to that obtained with ^{60}Co. A better definition and a higher fluorescence intensity of comets was obtained with fixation in ethanol before staining with ethidium bromide, in agreement with the results obtained by Woods et al. [26].

In contrast to the cytogenetic results, the comet assay showed that ^{90}Sr β-radiation was more efficient in inducing DNA damage than ^{60}Co γ-rays, when the cells were analyzed just after exposure. One of the possible hypotheses to explain this result may be related to the nature of the radioinduced lesions analyzed by two different methods. The comet assay allows us to detect unrepaired initial lesions in cells, such as single and double strand breaks and alkali label sites. But many of these primary induced lesions are repaired some minutes (4–15 min) [42,43] to some hours (2–3 h) after exposure [25,44] although above a certain level of exposure the cells can initiate a process of death as a consequence of unrepaired damage or erroneous repair and so, they will be selectively eliminated from the cell population. Among various lesions induced by ionizing radiation, double strand breaks (dsb) are considered to be the most difficult to repair: unrepaired or erroneously repaired lesions can be expressed as acentric fragments and dicentrics, respectively, visualized in mitoses.

Although the cellular death mechanism induced by ionizing radiation is not totally elucidated, an association between cellular reproductive death and dsb induction leading to the formation of chromosome aberrations has been reported by Lloyd et al. [3] and Bryant [45]. Cell death may be caused by loss of genetic material (acentric fragments) or by formation of chromosome bridges during anaphase (dicentrics), preventing the physical separation of daughter cells.

Ward [46] proposed that the ability to repair dsb can be influenced by the presence of other too close lesions such as multiple damage sites (MDS). These sites may be the result of a distinct radiation energy deposition pattern on DNA and differ from one type of radiation to another. So, as LET increases, the average energy deposited in the tissue increases, as also does the proportion of MDS. Due to the particulate nature and the low tissue penetration power compared to the other types of low LET radiation, ^{90}Sr β-radiation probably produced a greater complexity of lesions in the local DNA, resulting in a more difficult repair of dsb and increasing the incidence of cellular death.

As a matter of fact, the analysis of comet distribution (Fig. 4, Table 8) showed that cells irradiated with ^{90}Sr were more severely damaged than those irradiated with ^{60}Co and so more susceptible to be eliminated from the cell population. A possible selective death of highly damaged cells may be responsible in part for the low number of metaphases in blood samples irradiated with higher ^{90}Sr doses (3–5 Gy).

Cell elimination by apoptosis is one possibility, although further investigation is necessary. It is known that many cell types can develop an apoptotic process after exposure to physical and chemical agents. In the

case of lymphocytes, the induction of apoptosis by ionizing radiation has been reported both in vivo [47] and in vitro [48]. On the other hand, other modalities of interphase cell death that can contribute to rapid cell elimination after irradiation, or even other cellular processes like mitotic arrest leading to a lower incidence of chromosome aberrations cannot be excluded.

In summary, the two techniques applied in this study proved to be complementary, permitting the evaluation of DNA damage at different times after exposure. Chromosome aberration analysis provided rich information and the comet assay, for its simplicity and rapid execution, proved to be adequate to study early cell damage before the biological process of enzymatic repair.

Nevertheless, further investigations are necessary using others methodologies for a better analysis of the nature of radioinduced lesions in DNA and of cell repair, and also for a better understanding of the radiobiological phenomena involved.

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