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ORIGINAL PAPER

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Genotoxic and cytotoxic effects of ^{60}Co γ-rays and ^{90}Sr/^{90}Y β-rays on Chinese hamster ovary cells (CHO-K1)

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Abstract Among various types of ionizing radiation, the beta emitter radionuclides are involved in many sectors of human activity, such as nuclear medicine, nuclear industries and biomedicine, with a consequently increased risk of accidental, occupational or therapeutic exposure. Despite their recognized importance, there is little information about the effect of beta particles at the cellular level when compared to other types of ionizing radiation. Thus, the objective of the present study was to evaluate the genotoxic and cytotoxic effects of ^{90}Sr/^{90}Y—a pure, highly energetic beta source—on Chinese hamster ovary (CHO) cells and to compare them with data obtained with ^{60}Co. CHO cells irradiated with different doses of ^{60}Co (0.34 Gy min^{-1}) and ^{90}Sr/^{90}Y (0.23 Gy min^{-1}) were processed for analysis of clonogenic death, induction of micronuclei (MN) and interphase death. The survival curves obtained for both types of radiation were fitted by the exponential quadratic model and were found to be similar. Also, the cytogenetic results showed similar frequencies of radio-induced MN between gamma and beta radiations and the MN distribution pattern among cells did not follow the expected Poisson probability pattern. The relative variance values were significantly higher in cells irradiated with ^{90}Sr/^{90}Y than with ^{60}Co in all exposure doses. The irradiated cells showed more necrotic cells 72 h and 96 h after exposure to beta than to gamma radiation. In general, the ^{90}Sr/^{90}Y β-radiation was more damaging than ^{60}Co γ-rays. The data obtained also demonstrated the need to use several parameters for a better estimate of cellular sensitivity to the action of genotoxic agents, which would be important in terms of radiobiology, oncology and therapeutics.

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Introduction

Cancer is the result of the accumulation of genetic and epigenetic alterations involving genes that, directly or indirectly, control important biological functions such as cell proliferation and cell death [1].

Ionizing radiation is a physical agent known to induce mutation and cancer which, however, is also used as a widespread therapeutic modality for cancer treatment. Thus, one of the challenges in radiobiology and oncology is to understand how the cells respond to oxidative stress resulting from exposure to radiation and the pathway they will follow, i.e., whether they will die by an apoptotic or necrotic process or will survive and proliferate or not. These alternative pathways have profound implications for the whole organism.

It is well known that cell exposure to radiation results in direct and indirect DNA damage (base alteration, DNA-DNA and DNA-protein cross-linking, single-strand and double-strand breaks), and the extent of damage will depend on the type of radiation and the dose applied, among other factors. These phenomena occur because the deposition of radiation energy and its distribution do not hit cell organelles in a homogenous manner, generating multiple damaged sites in the DNA molecule [2]. These multiple damaged sites consist of locally accumulated individual lesions including double-stranded DNA damage of varying degrees of complexity, base damage and/or strand breaks and others. The higher the ionization density, i.e., the higher the radiation LET, the greater the complexity of the lesions and therefore, repair of the induced lesions is more difficult.

Studying the effects of beta radiation at the cellular level is of particular interest for direct application to nuclear medicine. Many radiopharmaceuticals used for diagnosis and therapy are beta emitters (e.g. ^{153}Sm-EDTMP, Na^{131}I, ^{186}Re-HEDP, ^{89}SrCl), which are con-

sidered to be the most promising ones because of their short tissue penetration (a maximum of 1–12 mm in soft tissue) and their energy (maximum 0.3–2.3 MeV), minimizing the radiotoxicity to surrounding healthy tissue [3, 4]. In addition, beta radiation is used in other fields such as nuclear industries and biomedicine, and is associated with occupational or accidental exposure [5].

Despite the recognized importance of beta radiation, few studies on its effects are available in the literature. Cytogenetic studies of ^{3}H [6, 7, 8], ^{131}I [9, 10, 11], ^{89}Sr [12] and ^{153}Sm [13, 14] have been conducted on human peripheral blood lymphocytes. Genotoxic effects of $^{90}Sr/^{90}Y$, a highly energetic pure beta particle source (E_{max}=2.27 MeV, E_{avg}=1.13 MeV) [15] were reported by Vulpis & Scarpa [16], Hall & Wells [17], Mill et al. [15] and Oliveira et al. [18] using the analysis of chromosome aberrations, micronuclei and comets in human blood samples.

Within this context, Chinese hamster ovary cells (CHO) have been used as a suitable biological system for genotoxic and cytotoxic studies because of their inherently advantageous characteristics such as belonging to a genetically stable cell line and having the ability to form colonies, a relatively rapid growth rate with a cell cycle of 12–14 h and a karyotype of 22±2 chromosomes [19].

Many studies with CHO cells have been conducted to assess the cytogenetic effects of gamma radiation [20, 21, 22], x-rays [23, 24, 25], ^{125}I [26, 27], alpha particles [28, 29], and neutrons [30, 31]. However, there are few studies on beta particles, such as those by Dikomey and Franzke [32] and Dikomey [33] who analyzed cell death and DNA breaks in CHO cells exposed to ^{3}H thymidine. There are no data in the literature about the effect of $^{90}Sr/^{90}Y$ on CHO cells.

In order to study the cellular radiosensitivity, Fenech et al. [34] proposed the integration of genotoxic and cytotoxic tests in the micronucleus assay to obtain more precise information about the action of the aggressive agent. The authors adopted morphological criteria for the identification of, and discrimination between, necrotic and apoptotic cells in the analysis of the cytotoxic effect of H_2O_2 on human lymphocytes, and for genotoxic evaluation. Both types of cells, necrotic and apoptotic represent a modality of cell death known as interphase death consisting of loss of physical and metabolic cell integrity [35].

On the basis of the above considerations, the present study was conducted in order to assess the genotoxic and cytotoxic effects of $^{90}Sr/^{90}Y$ β-rays on CHO cells and to compare them to the effects of ^{60}Co γ-rays. For this purpose, clonogenic death, micronucleus induction, and interphase death were analyzed and correlated as the main parameters. The choice of these parameters was justified by their biological importance, in addition to the fact that they are readily observable and measurable in irradiated cells. Micronuclei are related to irreparable damage, specifically DNA double-strand breaks, and to many late pathological alterations, such as cancer, and their determination is a relatively simple, rapid and sensitive technique. The advantages of the micronucleus assay were reported by Kirsch-Volders et al. [36] and Fenech et al. [34].

Considering these facts, the present study is intended to contribute to a better understanding of the effects of ionizing radiation, specially about $^{90}Sr/^{90}Y$ β-rays on the cell response, with important radiobiologic and oncologic implications.

Materials and methods

Cell line

CHO-K1 cells, subclones of Chinese hamster ovary cells (*Cricetulus griseus*), were maintained in culture flasks with RPMI 1640 medium (Gibco) supplemented with 10% fetal calf serum (Cultilab), 1% penicillin and streptomycin (Sigma), and incubated at 37°C in the presence of 5% CO_2.

Irradiation conditions

CHO-K1 cells in the exponential growth phase in PBS were exposed to ^{60}Co gamma radiation of the panoramic type (Yoshizawa Kiko, dose rate of 0.34 Gy min^{-1}) in Eppendorf tubes, at doses of 1.0, 2.5, 5.0, 7.5 Gy, at room temperature and in the presence of molecular O_2. The source was calibrated using a Fricke/Alanine dosimeter (Merck) and read in a spectrophotometer (HITACHI 100-40).

For irradiation with beta particles with the same doses, a $^{90}Sr/^{90}Y$ source (E_{max}=2.27 MeV, E_{avg}=1.13 MeV, Amersham Buchler, dose rate of 0.23 Gy min^{-1}) was adapted and mounted considering sterile irradiation conditions and low penetration of beta particles. The apparatus was made of acrylic, a material with a density close to that of water and similar to that of biological tissues and with a low atomic number that prevents the production of secondary x-rays when interacting with beta particles. The irradiation system and physical dosimetry were supervised by the Departamento de Metrologia das Radiações from IPEN-CNEN/SP (São Paulo, Brazil). The source was calibrated using an extrapolation chamber (23391 PTW, Freiburg). The cells suspended in PBS were placed on 7 mm high nylon dishes measuring 25 mm in diameter, forming a fine layer of approximately 1 mm and were irradiated with different doses of β-radiation.

Colony formation

To assess the clonogenic potential of gamma and beta radiation, irradiated cells (300 cells/plate) were seeded on ^Petri dishes (60 mm in diameter) for the analysis of colony-forming capacity. After 10–14 days, the medium was removed, the cells were washed with PBS, fixed with 10% formol for 30 min, and stained with 20% Giemsa solution for 10 min. The number of colonies was analyzed with a CP 600 colony counter (Phoenix). Small colonies with less than 50 cells were not considered. Three assays were carried out in triplicate for each dose and type of radiation.

The results were expressed as plating efficiency (PE) and survival fraction (SF), as described by Hall [37]: PE=(number of counted colonies/number of seeded cells)×100 and SF=(number of counted colonies in irradiated culture/number of seeded cells)×(PE in unirradiated culture/100).

Micronucleus test

To assess the genotoxic effects of gamma and beta radiation, the micronucleus test was carried out by the cytokinesis block method using cytochalasin B.

The procedure described by Guo et al. [38] to obtain binucleated cells was used. After irradiation with ^{60}Co and ^{90}Sr/^{90}Y, 3–4×10^4 cells were seeded on Petri dishes. Cytochalasin B (2 μg/ml) was added immediately after the irradiations. After 48 h of culture at 37°C with 5% CO_2, cells were trypsinized, transferred to 15 ml Falcon tubes, washed with PBS, and fixed with acetic acid and methanol (1:3). Finally, the cell suspensions were added drop-wise to histological slides and fixed at 65°C in a humid atmosphere, and stained with 10% Giemsa in phosphate buffer, pH 6.8.

Micronuclei with a diameter of less than one-third of the main nucleus, with a similar staining pattern as the main nucleus and no contact with it, were counted only in preserved cytoplasm, on the same focal plane.

The slides were examined with a Carl Zeiss microscope at ×400 magnification. For each dose, 500 binucleated cells containing up to 5 micronuclei were analyzed for 3 independent assays. All accompanying mononucleated and multinucleated cells were counted.

For the determination of the proliferation index the formula described by Eresxon et al. [39] was used: PI=[(number of mononucleated cells)+2(number of binucleated cells)+3(number of multinucleated cells)]/100.

Detection of apoptosis and necrosis

To assess the cytotoxic effects of ionizing radiation, the differential staining method described by Gorman et al. [40], which is based on the cell capacity to incorporate fluorescent DNA dyes was used. The evaluation was done 48, 72 and 96 h after ^{60}Co and ^{90}Sr/^{90}Y exposure with different doses of radiation (1.0, 2.5, 5.0, 7.5 Gy).

Stock solutions of acridine orange (100 μg/ml) and ethidium bromide (100 μg/ml) were mixed (v/v 1:1) and 1 μl of the final solution was added to 25 μl of the cell suspension (10^6 cells/ml in PBS). After 2 min at room temperature, 10 μl of this cell suspension was dropped on previously siliconized histological slides (2% dimethyldichlorosilane solution in 1,1,1-trichloroethane, LKB, Sweden), covered with coverslips and observed with a fluorescence microscope (Carl Zeiss) equipped with an excitation filter (DAPI/FITC/Texas red) and a barrier filter (TBP) at ×400 magnification.

The apoptotic cells in the initial and final phases were stained green and orange, respectively, both with chromatin condensation, whereas necrotic cells were stained in orange with organized chromatin. The green cells result from the penetration of acridine orange through the plasma membrane and orange cells are permeable to the dyes, acridine orange and ethidium bromide, as a consequence of the loss of membrane permeability. For each radiation type 2 or 3 independent assays were carried out and 500 cells were analyzed per dose.

Statistical analysis

The survival curves for CHO-K1 cells irradiated with ^{60}Co and ^{90}Sr/^{90}Y were fitted by an exponential quadratic model, $Y = e^{-aD-bD^2}$, where Y is the survival fraction, a and b are model constants, and D is the absorbed dose in Gy.

The dose-response curves for MN induction and cell death were fitted by a linear regression model, Y=c+aD, where c is model constant, and Y is the frequency of MN and cell death. The slopes of the adjusted curves were compared with the Student's t-test. Values of $p<0.05$ were considered significant.

The distribution of MN in binucleated cells were examined with a standard u-test, described by Papworth, as adapted by Savage [41], and used to evaluate the data according to Poisson probabilities. The variance to the mean ratio (σ^2/Y), and the dispersion index (u) values were determined. Overdispersion was considered significant ($p<0.05$) when the value of u exceeded 1.96. Values of $\sigma^2/Y<1$ indicate underdispersion while values >1 indicate overdispersion. All analyses were performed with the GraphPad Prism program (version 2.00).

Results

Colony formation

The data obtained for the clonogenicity of CHO-K1 cells irradiated with ^{60}Co and ^{90}Sr/^{90}Y are presented in Table 1. CHO-K1 cells showed relatively high plating efficiency (about 90%) before irradiation.

Figure 1 shows the survival curves for ^{60}Co and ^{90}Sr/^{90}Y obtained in the colony formation test, with a characteristic shoulder at low doses and a tendency to an exponential with increasing doses. Both curves were fitted by an exponential model:

$$Y = e^{\left[-(0.195\pm0.061)D-(0.014\pm0.014)D^2\right]} \text{ for } {}^{60}\text{Co}(r^2 = 0.92)$$

and

$$Y = e^{\left[-(0.215\pm0.036)D-(0.008\pm0.008)D^2\right]} \text{ for } {}^{90}\text{Sr}/{}^{90}\text{Y}(r^2 = 0.96),$$

and were considered statistically equivalent $(p > 0.05)$.

Micronucleus test

The data concerning the distribution and frequency of radio-induced MN in binucleated cells are shown in Table 2 and Fig. 2. The dose-response curves for ^{60}Co and

Table 1 Plating efficiency and survival fraction of CHO-K1 cells after the exposure to ^{60}Co and ^{90}Sr/^{90}Y

Type of radiation	Dose	Number of counted colonies	Plating efficiency (%)	Survival fraction
	(Gy)	(Mean±SD)	(Mean±SD)	(Mean±SD)
^{60}Co γ-radiation	0.0	266.7±22.0	88.9±7.3	1.00±0.00
	1.0	211.6±27.5		0.80±0.15
	2.5	146.0±35.0		0.55±0.14
	5.0	81.2±39.2		0.31±0.16
	7.5	14.0±0.9		0.05±0.01
^{90}Sr/^{90}Y β-radiation	0.0	270.9±1.0	90.3±0.3	1.00±0.00
	1.0	201.8±12.7		0.75±0.05
	2.5	152.9±27.6		0.56±0.10
	5.0	92.7±6.1		0.34±0.02
	7.5	18.0±1.7		0.07±0.01

SD Standard deviation

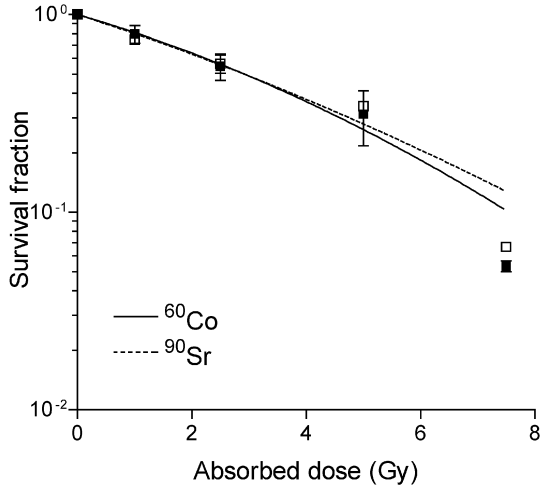

Fig. 1 Survival curves of CHO-K1 cells irradiated with ^{60}Co (*continuous line*) and ^{90}Sr/^{90}Y (*dashed line*). The curves were adjusted by exponential model ($Y = e^{-aD-bD^2}$)

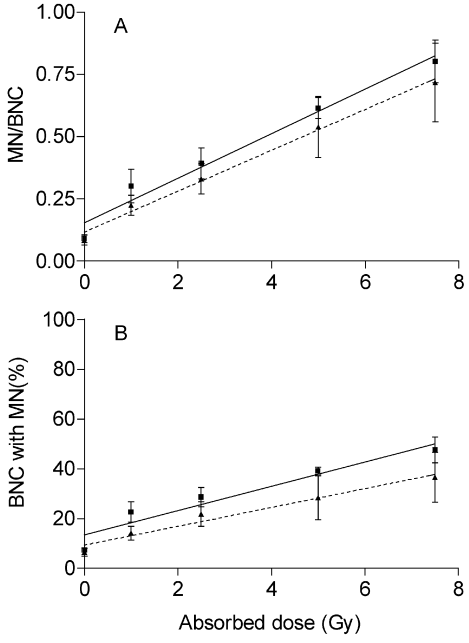

Fig. 2A, B Dose-response curves adjusted by linear regression model for micronuclei induction in CHO-K1 cells after exposure to ^{60}Co (*continuous line*) and ^{90}Sr/^{90}Y (*dashed line*). **A** Number of micronuclei per binucleated cell, **B** percentage of binucleated cells with micronuclei

^{90}Sr/^{90}Y were best fitted by the linear regression model. The frequency of MN increased as a function of radiation dose in cells irradiated by gamma as well as beta radiation. The coefficients and *r* values of the model are presented in Table 3, indicating that the data adequately fitted the model. Statistical analysis showed no significant difference between the effects of the two types of radiation ($p>0.05$).

An analysis of MN distribution obtained in samples exposed for both types of radiation (Table 2) showed that the value of *u* ranged from 8.45 to 11.91 for γ-radiation and from 9.80 to 18.83 for β-radiation, with all values being positive and greater than 1.96, which showed an overdispersion and hence deviated from a Poisson distribution. The σ^2/Y values varied from 1.314 to 1.434 for γ-rays and from 1.356 to 1.678 for β-rays. It can be observed that the basal values of σ^2/Y and *u* were similar to cells irradiated with γ-radiation and β-radiation. For γ-radiation, the σ^2/Y and u-values did not change significally with dose, whereas for β-radiation, the corresponding values with applied doses were higher and statistically different as indicated by the *t*-test ($p<0.05$).

The proliferation index calculated from the frequency of mononucleated, binucleated, and multinucleated cells showed an oscillation from 1.55 to 1.68 for γ-radiation and from 1.54 to 1.60 for β-radiation. There were no alterations in the proliferation kinetics in function of the dose for both types of irradiation.

Table 2 Frequency and distribution of micronuclei (MN) in binucleated CHO-K1 cells (BNC) after exposure to ^{60}Co and ^{90}Sr/^{90}Y

Type of radiation	Dose	Total of BNC analyzed	Binucleated cells with MN					Total of MN	Number of BNC with MN	σ^2/Y	U
	(Gy)		1	2	3	4	5	(MN/BNC±SE)	(%±SE)		
^{60}Co γ-radiation	0.0	1502	86	19	4	0	0	136 (0.091±0.008)	109 (7.26±0.70)	1.365	10.05
	1.0	1508	251	68	20	2	0	455 (0.302±0.014)	341 (22.61±1.22)	1.314	8.62
	2.5	1433	288	96	18	5	2	564 (0.394±0.017)	409 (28.54±1.41)	1.316	8.45
	5.0	1508	364	155	49	14	10	927 (0.615±0.020)	592 (39.26±1.61)	1.434	11.91
	7.5	1506	375	235	76	19	12	1209 (0.803±0.023)	717 (47.61±1.78)	1.350	9.61
^{90}Sr/^{90}Y β-radiation	0.0	1501	82	19	1	1	0	127 (0.085±0.008)	103 (6.86±0.68)	1.356	9.80
	1.0	1540	150	53	20	7	0	344 (0.223±0.012)	230 (14.94±0.98)	1.678	18.83
	2.5	1520	242	84	25	3	1	502 (0.330±0.015)	355 (23.36±1.24)	1.415	11.44
	5.0	1511	259	151	54	14	7	814 (0.539±0.019)	485 (32.10±1.46)	1.609	16.74
	7.5	1443	353	164	65	21	11	1015 (0.703±0.022)	614 (42.55±1.72)	1.469	12.60

Poisson distribution: u>1.96 overdispersion, $p<0.05$.
σ^2/Y Relative variance.
u Dispersion index.
SE Standard error.

Table 3 Coefficients of linear regression model for dose-response curves of micronucleus (MN) induction in CHO-K1 binucleated cells (BNC) after exposure to ^{60}Co and ^{90}Sr/^{90}Y

Type of radiation	Parameter	c (±SE)	a (±SE)	r
^{60}Co γ-radiation	MN/BNC	0.15±0.04	0.09±0.01	0.93
	BNC with MN (%)	13.44±2.66	4.89±0.63	0.91
^{90}Sr/^{90}Y β-radiation	MN/BNC	0.12±0.06	0.08±0.01	0.85
	BNC with MN (%)	9.38±4.01	3.78±0.95	0.74

Student's *t*-test, $p>0.05$, ^{60}Co versus ^{90}Sr/^{90}Y
SE Standard error

Table 4 Frequency of viable, necrotic and apoptotic cells evaluated at different times by differential staining technique in CHO-K1 cells after exposure to ^{60}Co and ^{90}Sr/^{90}Y

Type of radiation	Time of incubation	Dose	Total of analyzed cells	% Viable cells	% Necrotic cells	% Apoptotic cells
	(h)	(Gy)		Mean±SD	Mean±SD	Mean±SD
^{60}Co γ-radiation		0.0	1011	80.2±1.8	18.2±2.7	1.6±0.8
	48	1.0	1012	79.9±6.2	18.6±6.4	1.5±0.1
		2.5	1013	75.6±4.1	23.7±4.7	0.8±0.6
		5.0	1003	71.9±4.2	27.5±3.7	0.6±0.6
		7.5	1009	64.7±10.2	34.1±9.6	1.2±0.6
		0.0	1025	83.2±0.6	16.6±0.8	0.2±0.3
	72	1.0	1013	77.2±3.0	22.4±3.0	0.4±0.0
		2.5	1010	73.3±3.5	26.1±3.9	0.6±0.4
		5.0	1017	71.2±5.3	27.7±4.7	0.9±0.6
		7.5	1011	69.9±4.7	29.8±4.5	0.3±0.1
		0.0	1015	79.4±5.1	20.0±0.8	0.6±0.4
	96	1.0	1013	74.9±4.3	25.0±3.0	0.1±0.1
		2.5	1016	72.2±6.3	27.1±3.9	0.7±0.3
		5.0	1015	68.8±5.0	30.5±4.7	0.7±0.4
		7.5	1016	64.8±2.1	34.7±4.5	0.5±0.0
^{90}Sr/^{90}Y β-radiation		0.0	1521	80.8±2.2	19.1±2.0	0.1±0.2
	48	1.0	1521	76.9±4.2	22.6±4.3	0.5±0.1
		2.5	1523	73.1±4.1	26.7±3.8	0.2±0.3
		5.0	1521	67.5±3.5	31.8±3.7	0.7±0.4
		7.5	1533	62.4±4.5	36.6±4.5	1.0±0.2
		0.0	1518	82.1±3.1	17.9±3.1	0.0±0.0
	72	1.0	1519	74.5±4.3	25.1±4.3	0.4±0.2
		2.5	1523	68.4±1.9	31.2±1.4	0.4±0.5
		5.0	1517	62.6±1.4	36.7±1.7	0.7±0.5
		7.5	1526	56.9±1.3	41.9±1.4	1.2±0.9
		0.0	1521	80.3±4.0	19.0±4.2	0.7±0.3
	96	1.0	1527	73.7±3.0	25.6±3.8	0.7±0.5
		2.5	1530	68.8±4.5	30.6±4.7	0.6±0.4
		5.0	1523	63.6±5.7	35.2±5.9	1.2±0.5
		7.5	1528	55.7±6.8	43.2±5.6	1.1±0.6

SD Standard deviation.

Differential staining

This experiment was carried out to assess cell death in irradiated CHO-K1 cells using differential staining techniques. Table 4 summarizes the values of viable, necrotic and apoptotic cells obtained for irradiated samples as a function of the incubation time. Cell viability gradually decreased with increasing doses of ^{60}Co and ^{90}Sr/^{90}Y, while the number of necrotic cells increased. In contrast, with respect to apoptosis, only a small fraction of irradiated cells died (<1.5%) by this process. These data indicate that apoptosis is not the main death modality in these irradiated cells.

The basal values of necrotic cells were similar at different incubation times in both types of radiation. The statistical analyses showed no difference among different incubation times ($p>0.05$), indicating that the incubation period did not interfere with cell viability.

Figure 3 shows the dose-response curves adjusted for necrosis induction by the two types of radiation at different times after exposure (48, 72 and 96 h). The coefficients of the adjusted curves are presented in Table 5.

The CHO-K1 cells irradiated with beta particles showed more necrotic cells 72 and 96 h after exposure than with γ-radiation ($p<0.05$), but no significant difference was observed after 48 h ($p>0.05$).

Figure 4 shows the relationship between micronucleated and necrotic cells 48 h after exposure to ^{60}Co and ^{90}Sr/^{90}Y. The values of correlation coefficient (r) showed a positive correlation between these parameters (Table 6). As shown in Fig. 4, the beta radiation induced more cell death than gamma radiation, considering the number of MN per cell (Fig. 4A) or the percentage of micronucleated cells (Fig. 4B). Therefore, the statistical analysis showed no significant difference between the beta and gamma radiation ($p>0.05$).

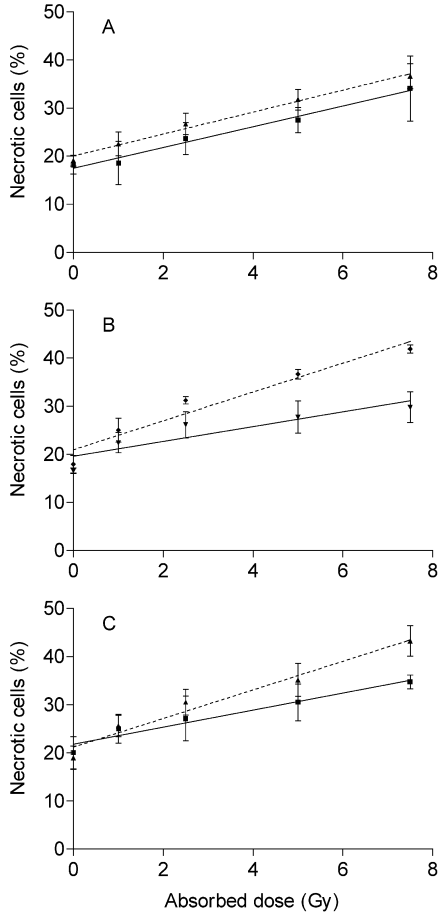

Fig. 3 Dose-response curves adjusted by linear regression model for necrosis induction, analysed after 48 h **A**, 72 h **B** and 96 h **C** in CHO-K1 cells after exposure to ^{60}Co (*continuous line*) and ^{90}Sr/^{90}Y (*dashed line*)

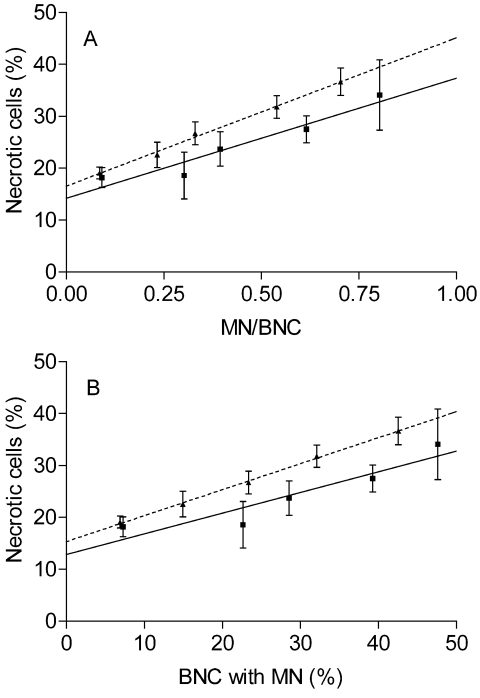

Fig. 4A,B Correlation between micronucleated and necrotic CHO-K1 cells 48 h after exposure to ^{60}Co (*continuous line*) and ^{90}Sr/^{90}Y (*dashed line*). **A** Micronucleus per binucleated cell versus percentage of necrotic cells, **B** percentage of binucleated cells with micronucleus versus percentage of necrotic cells

Discussion

In the present study, the cytotoxic and genotoxic effects of gamma and beta radiation were analyzed in CHO-K1 cells by cytogenetic assays, differential staining and colony formation.

Micronuclei represent unrepaired damage, more specifically, DNA double-strand breaks, and are involved in malformation, aging and cancer induction. Necrotic,

Table 5 Coefficient of adjusted curves for necrotic death by linear regression for ^{60}Co and ^{90}Sr/^{90}Y

Parameter	Type of radiation	Time of incubation (h)	c (±SE)	a (±SE)	r
Necrosis	^{60}Co γ-radiation	48	17.52±2.32	2.15±0.55	0.81
		72	19.62±1.78	1.54±0.42	0.79
		96	21.81±1.96	1.77±0.46	0.81
	^{90}Sr/^{90}Y β-radiation	48	20.05±1.35	2.29±0.32	0.89
		72	20.97±1.27	3.00±0.30	0.94
		96	21.21±1.83	2.97±0.43	0.88

Student's *t*-test, ^{60}Co versus ^{90}Sr/^{90}Y, $p>0.05$ for 48 h and $p<0.05$ for 72 h and 96 h after exposure.
SE Standard error.

Table 6 Coefficients of correlation for necrotic cells (%) and micronucleus induction (MN/BNC and BNC with MN (%)) in CHO-K1 cells after exposure to ^{60}Co and ^{90}Sr/^{90}Y

Type of radiation	Parameter	c (±SE)	a (±SE)	r
^{60}Co γ-radiation	MN/BNC x necrotic cells	14.22±3.25	23.12±6.42	0.79
	BNC with MN (%)×necrotic cells	12.85±3.86	0.40±0.12	0.76
^{90}Sr/^{90}Y β-radiation	MN/BNC×necrotic cells	16.57±1.72	28.56±3.93	0.89
	BNC with MN (%)×necrotic cells	15.37±1.85	0.50±0.07	0.89

Student's *t*-test, $p>0.05$, ^{60}Co versus ^{90}Sr/^{90}Y.
SE Standard error.

apoptotic and clonogenic deaths represent different cellular manifestations in response to an aggressive agent.

Various methodologies have been adopted to quantify cell death. Analyses based on morphological characteristics and on membrane permeability present the advantage of relative simplicity and rapidity, in addition to permitting the discrimination between the early and late stages of the cell death process [42, 43].

The data obtained showed that necrosis was the main modality of interphase death in irradiated CHO-K1 cells, in agreement with data reported by Fenech et al. [34] for human lymphocytes exposed to H_2O_2. The basal values of necrotic cells ranged from 16 to 20% after 48–96 h of incubation. Fenech et al. [34] observed a slightly higher basal value of about 23% in human lymphocytes exposed to H_2O_2 in vitro.

On the other hand, apoptotic death was rarely observed in irradiated CHO-K1 cells (<1.5%). These cells are known as defective in p53, a tumor suppressor gene, that plays a primordial role in inducing apoptosis in response to DNA damage, as well as in the control of cell cycle progression and the maintenance of mammalian genome integrity [44]. CHO-K1 cells present a mutation in codon 211 (exon 6) of the p53 gene, with alteration of Thr (ACA) to Lys (AAA) inside the DNA-binding region [44, 45] and this might be the reason for the low incidence of apoptosis. The p53-deficient cells are generally resistant to radio-induced apoptosis [43, 46].

Moreover, interphase death, another cell death modality, the reproductive one that consists of the loss of the ability to proliferate [35], was evaluated by the classical colony formation test. The survival curves for the two types of radiation were similar and were best fitted by the classical exponential quadratic model. The data showed that micronucleated and necrotic cells induced by ^{60}Co and $^{90}Sr/^{90}Y$ represent a significant fraction of the irradiated CHO cell population and are probably responsible for the exponential portion of the survival curve.

With respect to chromosome damage, the mean frequencies of spontaneous and induced MN were also similar for both types of radiation. In the analysis of MN distribution, a high overdispersion was observed in CHO-K1 cells for both types of radiation (non-Poisson distribution), with relatively high σ^2/Y and u-values in irradiated as well as in unirradiated samples.

We do not have a full explanation for the observed overdispersion, considering that the all samples were homogenously irradiated, and hence these factors may not contribute to the phenomenon.

These observations suggest an intrinsic sensitivity that can be characteristic of these cells. In other words, these phenotypic manifestations can be associated with genomic instability considering a cell line maintained for many generations in culture conditions. Elevated frequencies of MN could be related to an overall genetic instability [1]. It might explain, at least in part, the relatively high spontaneous rate of MN found in CHO-K1 cells (about 0.085–0.091 MN/BNC) in relation, for example, to that observed in human lymphocytes (about 0.002–0.036 MN/BNC) [47].

The relative variance values (σ^2/Y) did not change significantly with the dose for gamma radiation, whereas, for beta radiation, the values were significantly higher than gamma ones in all levels of exposure doses.

It is possible that beta radiation, because of its particulate nature and low tissue penetration power, causes a more complex lesion at multiple sites compared to electromagnetic radiation, leading to a damage which is difficult to repair, particularly DNA double-strand breaks, and highly responsible for various effects [48]. This might mean that beta particles show a greater probability to produce more DNA lesions per ionizing event than gamma rays.

A correlation analysis showed a close association between chromosomal damage and cell death. The statistical analysis showed no significant difference between the effects of gamma and beta radiations 48 h after exposure, in terms of both damaged cell proportions (percentage of micronucleated cells) and the extent of radio-induced damage (number of MN per cell) (Fig. 4). However, in addition to the DNA damage, other equally important lesions that probably do not contribute to the MN formation, are involved in cell death. Cell membrane lesions are indicated as a factor that can lead to cell death. Damage to the membrane structure can occur by free radicals ($OH^{\cdot}$, $H^{\cdot}$) and by reactive species such as $O_2^{-\cdot}$ and H_2O_2, originating from water radiolysis that are extremely oxidant, can react immediately with organic cell molecules, resulting in intracellular peroxides and lipid peroxides among others, affecting the structure and the physical and chemical properties of the membrane [49].

It is possible that these lesions (genetics and epigenetics) are produced in higher quantities by beta than gamma radiation, which could explain the higher frequency of necrotic cells obtained 72 h and 96 h after $^{90}Sr/^{90}Y$ exposure.

The MN assay also showed that under similar cell culture conditions there was no change in cell proliferation kinetics, at least within the ^{60}Co and $^{90}Sr/^{90}Y$ dose range applied.

In summary, the present work evaluated the effects of two types of low LET radiation, gamma and beta, and showed that, in general, $^{90}Sr/^{90}Y$ β-particles were more damaging than ^{60}Co γ-rays in CHO-K1 cells. Also, it is of fundamental importance to assess the cellular response in its various aspects by applying several correlated parameters for a better understanding of the global impact of an aggressive agent. Obviously, future investigation are needed using other methodologies involving morphological, biochemical and/or molecular approaches for a better evaluation of radiobiological phenomena. Monte Carlo calculations are planned in order to simulate beta and gamma interactions and verify possible reasons to explain why $^{90}Sr/^{90}Y$ β-particles were more damaging than ^{60}Co γ-rays in cells.

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