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INDUCTION OF CHROMOSOMAL ABERRATIONS IN HUMAN LYMPHOCYTES BY FISSION NEUTRONS

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ABSTRACT

Chromosome aberrations induced by sparsely ionizing radiation (low-LET) are well known and cytogenetic analyses of irradiated human lymphocytes have been widely applied to biological dosimetry. However, much less is known about chromosome aberrations induced by densely ionizing radiation (high LET), such as that of alpha particles or neutrons. Such particles induce DNA strand breaks, as well as chromosome breakage and rearrangements of high complexity. This damage is more localized and less efficiently repaired than after X- or γ-ray irradiation. This preferential production of complex aberrations by densely ionizing radiation is related to the unique energy deposition patterns, which produces highly localized multiple DNA damage at the chromosomal level. A better knowledge of the interactions between different types of radiation and cellular DNA is of importance, not only from the radiobiological viewpoint but also for dosimetric and therapeutic purposes. The objective of the present study was to analyse the cytogenetic effects of fission neutrons on peripheral blood lymphocytes in order to evaluate structural and numerical aberrations and number of cells in the different mitotic cycles. So, blood samples from five healthy donors, 22 – 25 years old, of both sexes, were irradiated in the Research Reactor IEA-R1 of our Institute (IPEN/CNEN-SP) with thermal and fast neutrons at doses of 0.2; 0.3; 0.5 and 1.0 Gy. The γ contribution to the total absorbed dose was about 30%. These doses were monitored by thermoluminescent dosimeters: LiF-600 (for neutrons) and LiF-700 (for γ-rays). The data concerning structural aberrations were evaluated with regard to three parameters: percentage of cells with aberrations, number of aberrations/cell and number of dicentric/cell. The cytogenetic results showed an increase in the three parameters after irradiation with neutrons, as a function of radiation dose. Apparently, there was no influence of neutrons on the kinetics of cellular proliferation.

1. INTRODUCTION

It is well known that all types of ionizing radiation induce qualitatively similar effects in biological systems. However, the damage severity and lesions persistence in the cells depend, essentially, of the type of radiation and of the applied dose. This occurs because biological effects are closely related to linear energy transfer (LET). In general, high-LET radiation (α-particles and neutrons) is more efficient in inducing biological damage than low-LET radiation (γ and X rays, β particle) for the same absorbed dose [1]. This phenomenon is essentially related to the pattern of deposition of radiation energy and its distribution in the matter, that in the case of high-LET radiation, generates multiple damaged sites in DNA of high complexity. Consequently, the repair of these lesions becomes slower and more difficult [2].

Among various types of ionizing radiation, the cytogenetic effects of X and γ rays are the best known and best characterized [3, 4]. In relation to neutron, little information is available, being however of great interest, especially in cases of accidents such as that occurred in Tokai-mura [5], the atomic explosions in Japan [6] in Sarov and Mayak in Russia [7], among others. Sometimes, accidental exposures include low- and high-LET radiations [8].

Among several effects observed in exposed individuals to neutron and γ radiation, a significant increase in the incidence of several types of cancer such as thyroid, lung, breast, leukemias and lymphomas have been reported [9].

With the continuing growth of nuclear industry, plant reactors for energy generation and the introduction of neutron sources in radiotherapy for slowly growing cancers, including brain, uterine cervix, prostate, head and neck tumors [10, 11], a better understanding of neutrons effects at the cell level becomes necessary [1, 8, 12].

The aim of this study is to assess the cytogenetic effects induced by mixed fission neutrons and γ-rays beam in human peripheral lymphocytes, by chromosome aberration technique, establishing a dose-response relationship. 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 [13].

2. MATERIAL AND METHODS

Blood samples were obtained from 5 healthy donors, 22 - 25 years, of both sexes, non-smokers, with no history of radiation exposure. The blood samples were fractionated and irradiated in the Research Reactor IEA-R1 with thermal (3 Gy/h) (0.5 eV) and fast neutrons (3 Gy/h) (17-20 MeV) at doses of 0.2; 0.3; 0.5 and 1.0 Gy. The γ-ray contamination was about 30% of the neutron dose. These doses were monitored by thermoluminescent dosimeters: LiF-600 (for neutrons) and LiF-700 (for γ-rays).

Blood cells were cultivated in RPMI 1640 (Cultilab), supplemented with 20% bovine foetal serum (Cultilab), 5μg/ml BrdU (Sigma) and stimulated with 5μg/ml phytohemaglutinin (Gibco) and maintained for 48 h at 37^{o}C. The cells were then treated with 0.7 μg/ ml colcemid (Sigma) for 2 h, hypotonized with 0.075 M KCl and 1% sodium citrate (Merck), fixed and spread on histological slides pre – heated at 65ºC.

For evaluation of cell cycle kinetics, the fluorescence plus Giemsa (FPG) staining technique that allows unequivocal identification of cells from first, second or later mitoses was utilized [14, 15]. The slides were stained with 5 μg / ml Hoechst 33258 (Sigma), covered with 0.5 ml McIlvaine buffer and exposed to UV light (254 nm) for 20 minutes at 60ºC on a heated plate.

They were then washed with distilled water and stained with 5% Giemsa (Sigma) in Sorensen buffer, pH 6.8, and analyzed under an optical microscope (Carl Zeiss, Germany).

All the metaphases containing diploid number of up to 2n-2 chromosomes were considered. The structural chromosome aberrations were classified according to the criteria established by the International Atomic Energy Agency [15] and were evaluated with regard to three main parameters: incidence of affected cells (percentage of cells with aberrations), degree of intracellular damage (number of aberrations/cell) and occurrence of dicentric chromosomes, a specific type of chromosome aberration.

The statistical analysis was done using the *GraphPad Prism* software. The cytogenetic data obtained were fitted to linear ($Y= \alpha D$) and linear-quadratic models ($Y= \alpha D + \beta D^2$), where Y is the frequency of chromosome aberrations, α the linear coefficient, β the quadratic coefficient, and D is the dose (Gy).

3. RESULTS

The cytogenetic analysis of human blood lymphocytes irradiated *in vitro* with neutron showed that the major types of structural chromosome aberrations observed were chromatidic/chromosomic gaps and breaks, acentric fragments, centric rings, double minutes and dicentrics.

The dose-response relationships for induction of chromosome aberrations are illustrated in Figure 1. The three parameters analyzed, i.e., percentage of cells with chromosome aberrations, number of aberrations/cell and number of dicentrics/cell increased as a function of the radiation dose (Table 1). The α and β coefficients of the models used to fit the curves with their respective standard error (S.E.) for the induction of dicentrics are presented in Table 2. Values of r^2 showed that the data fitted equally well with the linear ($r^2= 0.9976$) as with the linear-quadratic ($r^2= 0.9973$) model.

Table 1. Frequencies of structural chromosome aberration found in human lymphocytes irradiated *in vitro* with neutrons

Dose (Gy)	Number of cells scored	DIC	CR	DM	Break	Frag	CG	Number of aberr/cell (± SE)	Cell with aberr. (%) (± SE)	Number of dicentric/cell (± SE)
0.0	1600	07		04	03	04	02	0.015 ± 0.007	1.520 ± 0.667	0.0052 ± 0.0052
0.2	974	50	04	16	04	18	02	0.108 ± 0.028	9.837 ± 2.693	0.0567 ± 0.0122
0.3	856	59	02	20	09	30	05	0.147 ± 0.012	12.905 ± 0.742	0.0697 ± 0.0113
0.5	793	91	09	42	10	34	02	0.235 ± 0.026	18.590 ± 1.219	0.1150 ± 0.0067
1.0	1186	228	09	129	10	98	05	0.419 ± 0.049	30.020 ± 3.064	0.2470± 0.1136

DIC = dicentric; CR = centric ring; DM = double minute, CG = cromatidic and chromosome gaps, SE= standard errors

The frequencies of metaphases with a modal (2n= 46) and hypomodal (2n-1 and 2n-2) chromosome number varied from 90 to 96% in lymphocytes irradiated with neutron in the dose range analyzed. Similarly, the frequency of cells in second division was less than 10%.

Table 2. Values of α and β coefficients of linear and linear-quadratic model with respective standard errors (SE) for the induction of dicentrics in human peripheral lymphocytes irradiated with neutrons

Model	α (± SE)	β (± SE)	Dose range (Gy)	Reference
Linear	0.457 ± 0.046		0.005 – 0.5	[16]
	0.146 ± 0.016		0.104 – 0.527	[17]
	0.286 ± 0.021		0.2 – 1.0	Present study
Linear-quadratic	0.378 ± 0.074	0.275 ± 0.023	0.005 – 0.5	[16]
	0.195 ± 0.018	0.119 ± 0.200	0.0386 – 2.28	[14]
	0.181 ± 0.024	0.105 + 0.004	0.05 – 2.0	[18]
	0.242 ± 0.089	0.053+ 0.104	0.2 – 1.0	Present study

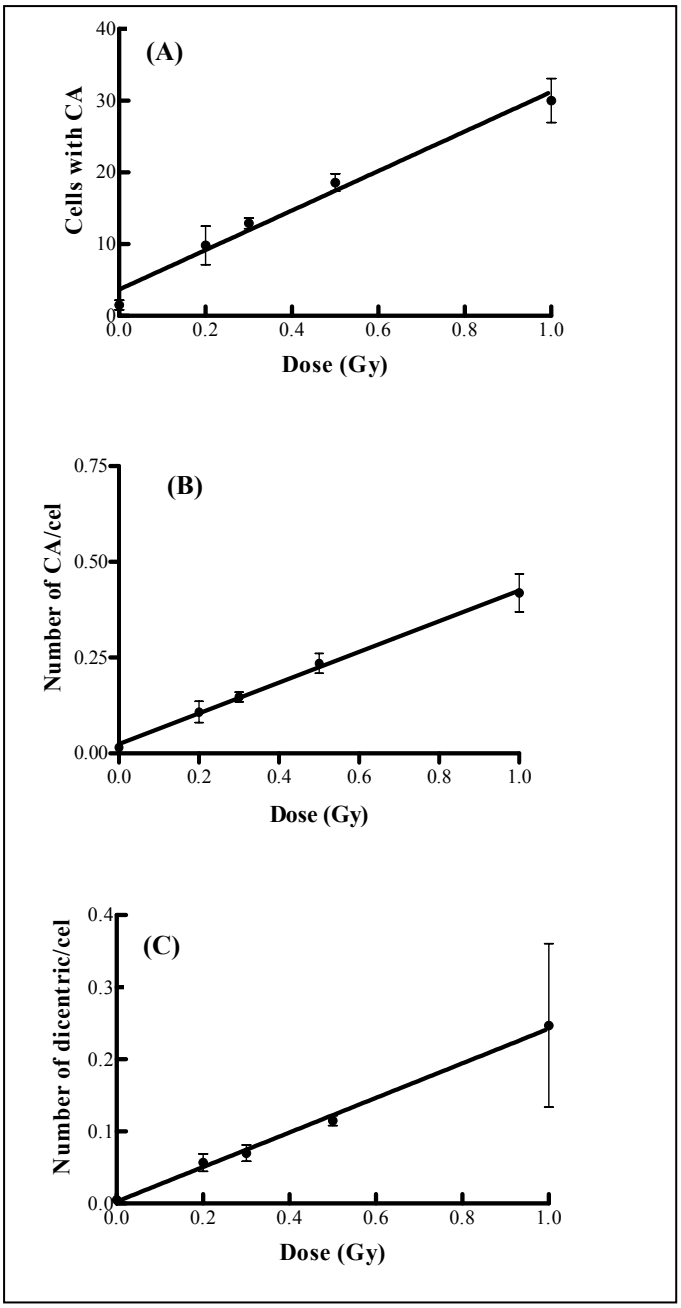

Figure 1. Dose-response curves adjusted by linear model (Y = αD). A) Percentage of cells with chromosome aberration; (B) number of aberrations/cell; (C) Number of dicentric/cell, obtained from peripheral lymphocytes exposed to neutrons. Standard errors are indicated in the curves

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.

In the present study we evaluated cytogenetic effect in the production of chromosome aberrations in human blood lymphocytes irradiated with a mixed beam of fission neutron and γ-ray. The fission neutrons are always mixed with gamma rays.

The cytogenetic data obtained showed various types of structural chromosome aberrations, the same types found in cells irradiated with low-LET radiation, as X- or γ-rays, β particles, in agreement with the observation that the effects of radiation are qualitatively but not quantitatively similar at the chromosomal level.

The dose-response relationships for X- and γ-radiation are fairly well documented, but few data are available for neutrons, and no comprehensive study has been made using a range of neutron energies [12].

Data from the literature indicate that for low-LET radiation, the cytogenetic data generally fit well the linear- quadratic model of dose-response, but for high-LET radiation a linear fit is more appropriate for all types of aberration. In the present study the dose response for dicentrics fitted both models, i.e., linear and linear-quadratic models which showed a best fit.

The biophysical interpretation of the linear-quadratic model for dicentric 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 [4, 19].

However, the quadratic coefficient of the dose-response curve obtained in the present study is even lower in relation to the linear coefficient and thus, the α/β ratio for dicentric was 4.6 Gy. This value is above the range of doses used in the present experiment (0.2 – 1.0 Gy). This suggests that at doses below this value in the dose-response curve, dicentrics are predominantly formed by one track only, inducing two breaks, and at doses above it the dicentrics are produced by two independent tracks. The higher α/β ratio for dicentrics (4.6Gy), suggests that dicentric chromosomes due to the two chromosomes breaks as a consequence of one track, appear predominant in the dose-response curve. These observations suggest the prevalence of the linear term and a smaller repair capacity after neutron irradiation [18], probably without dose rate effect.

Although the γ-ray contamination was about 30% of the neutron dose, it does not seem that this explains the existence of a quadratic component for neutrons, because of the relatively low efficiency of γ-rays compared to neutrons. This suggests that the biological effectiveness of the γ-ray component (about 30%) is not significant compared to that of neutrons [20]. Even for the reactor CRAC fission spectra neutrons with γ-contaminations up to 50%, a linear relationship has been reported [21].

Several authors have been reported that the occurrence of a quadratic component of the dose-response relationship for the production of dicentrics obtained in human lymphocytes by mixed fission neutron and γ-ray irradiation could be due to the effect of released fast recoil protons with low LET [20, 22] and not due to an additive effect of neutron and γ components in the production of dicentrics.

The data obtained also show that there was no difference in number of chromosomes or in frequency of cells in first or second division after neutron exposures. Thus, we suggest that neutron irradiation had no influence on modal chromosome number or on the cell cycle within the dose range analyzed.

5. CONCLUSION

Preliminary cytogenetic data, obtained in human blood lymphocytes irradiated with mixed beam of fission neutron and γ-ray, indicated the existence of a significant linear component (α) of the dose-response relationship for the production of chromosome aberrations, in the analyzed dose range. Because these two types of ionizing radiation present different biological effectiveness, the γ-ray contribution to the quadratic component (β) of the curve could be non significant.

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