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DOSE-RESPONSE CURVE FOR BLOOD EXPOSED TO GAMMA-NEUTRON MIXED FIELD BY CONVENTIONAL CYTOGENETIC METHOD

José Odinilson de C. Brandão[1,2], Priscilla L. G. Souza[1], Merilane S. Calixto[3], Joelan A. L. Santos [1,2], Neide Santos[3], Eudice C. Vilela[1] and Fabiana F. Lima[1]

[1] Centro Regional de Ciências Nucleares, CRCN - CNEN/NE
Av. Professor Luiz Freire 200
50740-540 Recife, PE
jodinilson@cnen.gov.br
fflima@cnen.gov.br

[2] Departamento de Energia Nuclear – Universidade Federal de Pernambuco, DEN/UFPE
Av. Professor Luiz Freire 1000
50740-540 Recife, PE
jasantos@cnen.gov.br

[3] Departamento de Genética – Universidade Federal de Pernambuco
Av. Professor Luiz Freire 1000
50740-540 Recife, PE
santos_neide@yahoo.com.br

ABSTRACT

There is increasing concern about airline crew members (about one million worldwide) are exposed to measurable neutrons doses. Historically, cytogenetic biodosimetry assays have been based on quantifying asymmetrical chromosome alterations (dicentrics, centric rings and acentric fragments) in mytogen-stimulated T-lymphocytes in their first mitosis after radiation exposure. Increased levels of chromosome damage in peripheral blood lymphocytes are a sensitive indicator of radiation exposure and they are routinely exploited for assessing radiation absorbed dose after accidental or occupational exposure. Since radiological accidents are not common, not all nations feel that it is economically justified to maintain biodosimetry competence. However, dependable access to biological dosimetry capabilities is completely critical in event of an accident. In this paper the dose–response curve was measured for the induction of chromosomal alterations in peripheral blood lymphocytes after chronic exposure *in vitro* to neutron-gamma mixes field. Blood was obtained from one healthy donor and exposed to two neutron-gamma mixed field from sources ^{241}AmBe (20 Ci) at the Neutron Calibration Laboratory (NCL – CRCN/NE – PE – Brazil). The evaluated absorbed doses were 0.2 Gy; 1.0 Gy and 2.5 Gy. The dicentric chromosomes were observed at metaphase, following colcemid accumulation and 1000 well-spread metaphase figures were analyzed for the presence of dicentrics by two experienced scorers after painted by giemsa 5%. Our preliminary results showed a linear dependence between radiations absorbed dose and dicentric chromosomes frequencies. Dose-response curve described in this paper will contribute to the construction of calibration curve that will be used in our laboratory for biological dosimetry.

1. INTRODUCTION

Biological dosimetry is one of the important research subjects in the field of radiation protection. The major concern is to estimate radiation dose by measurement of biological changes in exposed persons after irradiation. In addition, it may be possible to predict the future effect on health by a long-term follow-up study of radiation effects. A brief

introduction is given of the practices and the future aspects of research work in biological dosimetry using chromosome aberration analysis in this laboratory [1].

A significant number of people have the potential to be occupationally exposed over a protracted period to low doses of neutrons. There is increasing concern about airline crew members (about one million worldwide) are exposed to measurable neutrons doses [2].

Historically, cytogenetic biodosimetry assays have been based on quantifying asymmetrical chromosome alterations (dicentrics, centric rings and acentric fragments) in mytogen-stimulated T-lymphocytes in their first mitosis after radiation exposure [3].

Increased levels of chromosome damage in peripheral blood lymphocytes are a sensitive indicator of radiation exposure and they are routinely exploited for assessing radiation absorbed dose after accidental or occupational exposure. The dicentric assay technique in human peripheral blood lymphocytes has been shown as the most sensitive method of quantifying the radiation dose in the absence of physical measurements because of its ability to estimate the average whole-body dose [3].

Establishing a competent biodosimetry laboratory that is capable of performing cytogenetic analysis for dose estimation is of paramount importance in a country like ours, where large use of radioactive substances for peaceful purposes are in place. It has been suggested that each laboratory intended to carry out biological dosimetry should have its own in vitro dose–response calibration curve for dose reconstruction [4].

Since radiological accidents are not common, not all nations feel that it is economically justified to maintain biodosimetry competence. However, dependable access to biological dosimetry capabilities is completely critical in event of an accident [5].

In order to estimate a radiation dose absorbed during anaccident it is necessary a reference in vitro calibration curve. This curve is generated by irradiating blood samples, collected from control donors, with several doses of radiation [6].

The dose response curve for induction of exchange aberrations induced by low LET radiations is linear-quadratic, exemplifying contributions of both one and two track events and generally fits the equation:

$$Y = A + aD + bD^2 \qquad (1)$$

where Y is the yield of dicentrics, D is the dose, A is the background frequency, a is the linear coefficient and b is the dose-squared coefficient. With chronic exposure (low dose rate) to low LET radiation, the yield of dicentrics is linear. Following high LET radiation, the dose response for induction of dicentrics is predominantly linear.

This paper aimed to establish a dose-response curve based on dicentric assay through irradiation of blood samples by gamma-neutron mixed field.

2. MATERIALS AND METHODS

2.1 Blood samples

Heparinised blood (10 mL) was taken from one healthy individual, male, and 24 years old, at the Laboratory of Biological Dosimetry (LBD – CRCN/NE CNEN – PE – Brazil). The sample was divided equally (5mL + 5mL) between two culture tubes. One of them was irradiated and another kept at room temperature (~20 °C).

2.2 Irradiation

Blood was exposed to two mixed field neutron-gamma sources ^{241}AmBe (20 Ci) at the Neutron Calibration Laboratory (NCL – CRCN/NE – PE – Brazil). The distance between the sources and the sample was 3.75 cm (the centre of the sample was assumed to be the geometrical centre of the liquid in the tube, taking as main axis the one perpendicular to the beam direction). A polyethylene barrier (2mm) involving the tubes containing the samples was used to ensure the electronic equilibrium. Both of the sources were calibrated and the emission rate was determinate to be $(4.46 \pm 0.07) \times 10^6$ n/s in 03/15/2005. At the irradiation position, spectrum was determinate using a Bonner Sphere System manufactured by LUDLUM Measurements Inc., model 42-5. Immediately after exposure, both of the blood samples were cultured. The samples received doses of 0.20; 0.41 and 0.91 Gy of mixed field neutron-gama.

2.3 Cell culture

From each sample, two blood cultures were set up. Lymphocytes were cultured for 48 hours in RPMI 1640 media (Sigma), supplemented with 20% (v/v) fetal calf serum, 1% (v/v) hytohemaglutinin (Biological Insdustries), 1% Hepes (v/v) and 50 µg/mL streptomycin. 0,05 µg/mL Colcemid (Biological Industries) was added 46 hours after culture started. The cells were harvested by centrifugation of the samples and the cell pellets were resuspended in 0.075 M KCl and kept for 15 min at 37°C. After the hypotonic shock the cells were fixed 3 times in methanol:acetic acid (3:1). Finally, cells were dropped on clean slides and stained with a 5% Giemsa solution (Merk). One thousand well-spread metaphases were analyzed for the presence of dicentrics by two experienced scorers.

2.4 Scoring criteria

Scoring of chromosomal aberrations was performed directly at the optical microscope (Quimis Q708SK-5). Only cells at the first mitosis (colcemid blocked) were scored.

3. RESULTS AND DISCUSION

Koksal et al. [7] demonstrated that *in vitro* tests generate similar effects to those observed by exposure to radiation in vivo. Several calibration curves were constructed by using dicentrics and this model is universally accepted.

The person whose blood sample was used for irradiation with different doses not reported through the questionnaires, the existence of any criterion that makes it unfit to participate in

this study. This individual is a young adult, non-smoker who claims not to consume illegal drugs. In addition, the volunteer was not submitted to any procedure or diagnostic radiology during course of experimental activities and, still, before six months the begining. In sum, no factor was not detected in the questionnaire that could substantially change the results. The frequency of natural chromosomal alterations of the individual remained constant in the different phases in which samples were collected for irradiation (Table 1).

Table 1. Frequency of chromosomal changes in the samples not irradiated (control)

Control	N° de metaphases	N° of dicentrics	Dicentric frequencies
1	1000	1	0.001
2	1027	1	0.0009
3	1022	1	0.0009

For the construction of the calibration curve, it were used for counting net values observed in Table 2.

Table 2. Data used to fit the dose-response curve by CABAS

Dose (Gy)	N° of metaphases	Dicentric chromosomes
0.206	1000	27
0.413	1027	33
0.964	1022	95

These data were used in the computer program CABAS [5]. This software was developed specifically for biological dosimetry, and its objective is basically the determination of the adjustment parameters for the establishment of calibration curves based on chromosomal unstable alterations and/or micronuclei, in addition to the stable chromosomal changes viewable by FISH.

The experimental data obtained have led the curve shown in Figure 1.

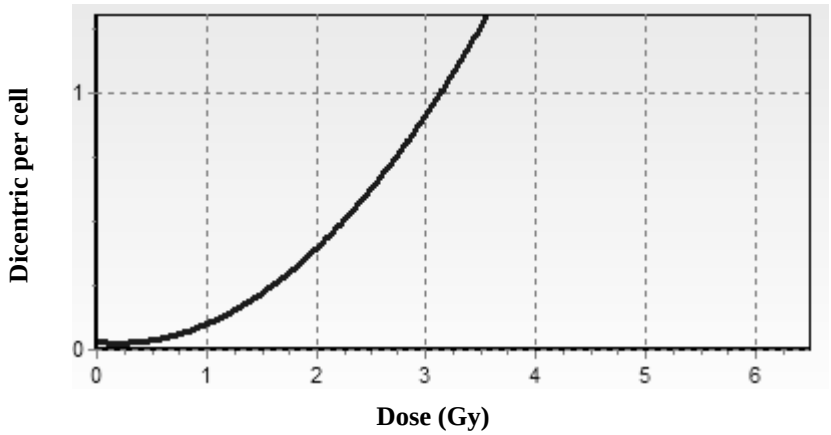

Figura 1. Dose-response curve generated by CABAS

The curve fit equation is expressed by:

$$Y = 0.0009 + 0.112\ D + 0.444\ D^2 \quad (2)$$

where Y is the frequency of chromosomal alterations and D is the dose in Gray (Gy).

These coefficients are comparable to those found by Lloyd et al. [8], where it was generated a calibration curve from the irradiation of blood samples by neutrons from a cyclotron. These neutrons had energies of 7.6 MeV and the curve fit equation was:

$$Y = 0.001 + 0.064D + 0.482D^2 \quad (3)$$

The difference observed in the linear coefficient is attributed to the difference in the composition of the irradiation field, since the source of ^{241}AmBe presents a gamma component, usually 1:1 on neutron [4], leading to greater production of chromosomal alterations induced by low-LET radiation.
The data obtained are in agreement with other already published and in use in some laboratories for biological dosimetry, which shows the possibility of using this calibration curve in case of radiological emergency.

4. CONCLUSIONS

This work enabled the establishment of a calibration curve for biological dosimetry in human peripheral blood lymphocytes to mixed neutron-gamma field. This curve is used exclusively in the Laboratory of Biological Dosimetry for the determination of absorbed dose in individuals occupationally exposed, or that perhaps, suffer accidental exposure to such radiation. Furthermore, this procedure could be routinely used to complement the physical dosimetry.

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