STUDY OF CHROMOSOME ABERRATIONS INDUCED IN HUMAN LYMPHOCYTES FOLLOWING IRRADIATION WITH ^{60}Co AND ^{137}Cs *IN VITRO*

Márcia Augusta da Silva*, Marisa Lemes* & Orlando Rebelo dos Santos*

*Instituto de Pesquisas Energéticas e Nucleares - IPEN-CNEN/SP
Caixa Postal 11049
05422-970, São Paulo, Brasil
masilva@net.ipen.br

ABSTRACT

The frequency of induced chromosomal aberrations in peripheral blood lymphocytes of exposed individual is currently being used as a valuable biological dosimeter to estimate the absorbed dose of ionizing radiation. It is the best biological indicator of radiation dose because of its easiness in identification and low background incidence. The dose is estimated by comparison of the observed yields of unstable chromosome aberration (dicentric and centric rings) with standard dose-response curves generated following *in vitro* irradiation of human lymphocytes. For calibration curve generation, blood samples from six healthy non-smoking adults (two females and two males) were submitted to irradiation process by ^{60}Co and ^{137}Cs gamma rays with doses in the range 0.2 - 4.0 Gy at a dose rate of 0.05 Gy.min.$^{-1}$ and as closely as possible to the *in vivo* situation. It was observed that the yield of dicentrics aberrations of the dose-response curve profile depended on the dose and the increment of this factor resulted in and increment in the yield of dicentrics per cell.

I. INTRODUCTION

The most fully developed biological method for dose assessment is cytogenetic dosimetry, which consists of chromosome aberrations analysis in the peripheral blood lymphocytes (PBL) of exposed persons to ionizing radiation. Dicentric and centric ring (unstable chromosome aberration) are the most frequently used in dosimetric procedures (1).

The use of calibration curve generated by other laboratories to estimate the dose absorbed by exposed individual can lead to significant uncertainties, so it is recommended by the International Agency Energy Atomic (IAEA) that all the cytogenetic dosimetry laboratories establish their own dose-response curves (2).

Chromosome aberrations induced in peripheral blood lymphocytes by exposure to ionizing radiation consist of a biological indicator very sensible to the alterations in quality and quantification of radiation. The peripheral lymphocytes are pre-synthetic phase of DNA, i.e. G_0 stage of cell cycle, and they form only chromosome-type aberration when exposed to ionizing radiation. The lymphocytes are the best biological indicators of radiation dose once they are distributed all over the body with a relatively long life; they respond to stimulation *in vitro* with phytohemaglutin (PHA) entering into mitosis; they are easily obtained and show a low incidence of spontaneous aberrations.

In 1962, Bender was the first researcher to propose that the frequency of radioinduced aberrations in human lymphocytes could be used as a biological method for dose assessment (4).

Since then, a large numbers of publications confirms the cytogenetic method as a useful dosimetric tool, defining many physical and biological factors which should have to be considered in the estimated of dose.

These experiments have demonstrated that the dose-response curve is generally fit the linear-quadratic equation: $Y=C+\alpha D+\beta D^2$, where Y is the frequency of the observed aberrations, C is the frequency of spontaneous aberrations, D is the dose, α and β are the regression coefficient, α is the coefficient of linear regression and β is the quadratic regression coefficient (3).

To low doses (less than 50 cGy) the probability that two tracks cross the target is extremely small and the dicentric will be produced almost exclusively by a track and of at low frequency. When the doses increases, the frequency of the dicentrics induced by two tracks will increase too.

The cytogenetic dosimetry is the most sensible system to the estimative of dose and when a sufficient number of metaphases is analysed, the estimative of dose corresponds of the whole body has high sensibility (inferior limit between 5-10 cGy to high radiations of low LET).

To high doses, under 800 cGy, the quantity of lymphocytes/mm^3 is low, by the way, very little lymphocytes will be capable of reaching mitosis and the dose-response curve tend to saturate.

II. MATERIAL AND METHOD

The blood samples were obtained by healthy donors, with age between 20 and 35 years old, both sexes, non-smoking and who weren't having any kind of medicine. The blood sample was collected in heparinised syringes by venous punction and kept on 37°C during all the irradiation procedure.

The samples were irradiated in source of ^{60}Co and in source ^{137}Cs in the doses of 20, 50, 100, 200, 300 e 400 cGy with dose rate of 5 cGy.min.$^{-1}$ and an aliquot was kept as a control. The cultive was effectuated in tubes of sterile culture. The sample was homogenises and 0,5 ml was dripped in a tube containing MEM supplemented with 20% of foetal calf serum, phytohemaglutinin and BrdU (both concentred on 5 mg/ml). The cultives were incubated for 46 hours in stove in 37°C and after this time was 0,1 mg/ml of colcemid added. The incubation was kept on for more two hours. After the period of cultive, the cells were hypotonized with KCl solution (0,075M) and sodium citrate on 1% (3:1) and fixed in methanol and acetic acid solution (3:1). The material was dripped onto dried slides, cleaned and kept on 65°C under UV light and covered with Mc Ilvaine buffer. After two hours, the slides were washed in distillater water and stained with Giemsa solution (Fluorescence plus Giemsa technique of Perry and Wolf, 1974).

III. RESULTS AND DISCUSSION

Stable and unstable aberrations are induced by ionizing radiation. The stable aberration aren't used in cytogenetic dosimetry by conventional technique of analisys of chromosome aberrations. The unstable aberrations, the dicentric is the best indication of the radiation damage, is easily identified and occurs with high frequency related to the other kinds of unstable aberrations (60%). The centric ring presents low frequency. The proportion between these aberrations is of 20:1. The frequency and the distribution of dicentrics and centric rings induced by ^{60}Co and ^{137}Cs with the dose-rate of 5 cGy.min^{-1} in the cells analysed in each dose and in 2 samples by radioactive treatment are presented in table 1 and 2 and plotted in figures 1 to 4.

Table 1. Distribution of dicentrics aberrations in peripheral blood lymphocytes after exposure to ^{60}Co *in vitro*

Dose	n° of cells	Dice	0	1	2	3	ring centric	Y (dic/cell)
0	1000	2	998	2	0	0	0	0.0020
20	1092	16	1076	16	0	0	0	0.0146
50	1000	38	962	38	0	0	0	0.0380
100	1000	92	910	86	3	0	1	0.0920
200	1000	220	794	185	16	1	4	0.2200
300	1001	359	701	240	52	5	3	0.3586
400	965	696	432	371	118	27	15	0.7212

Table 2. Distribution of dicentrics aberrations in peripheral blood lymphocytes after exposure to ^{137}Cs *in vitro*

Dose	n° of cells	Dic	0	1	2	3	ring centric	Y (dic/cell)
0	1000	0	1000	0	0	0	0	0.000
20	1162	24	1138	24	0	0	0	0.0206
50	1190	84	1110	76	4	0	0	0.0706
100	1100	113	998	84	13	1	4	0.1027
200	1000	262	764	210	23	2	1	0.2620
300	1000	385	668	282	47	3	0	0.3850
400	998	643	468	415	90	16	9	0.6443

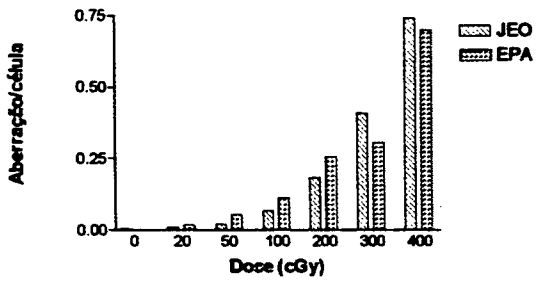

Figure 1. The distribution dicentrics of among cells in peripheral blood lymphocytes of each donor after exposure to ^{60}Co *in vitro*

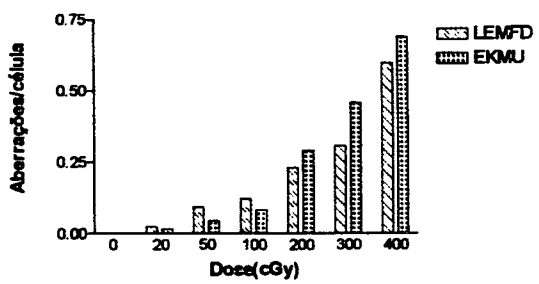

Figure 2. The distribution dicentrics of among cells in peripheral blood lymphocytes of each donor after exposure to 137Cs in vitro

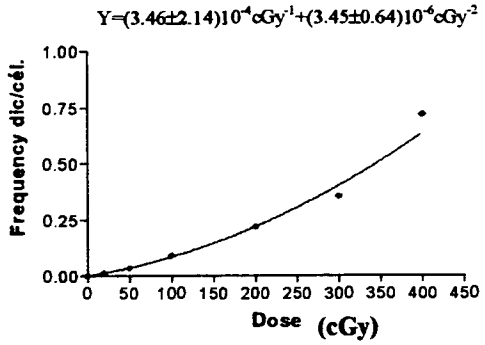

Figure 3. Frequency of chromosomal aberrations exposed to ^{60}Co *in vitro*

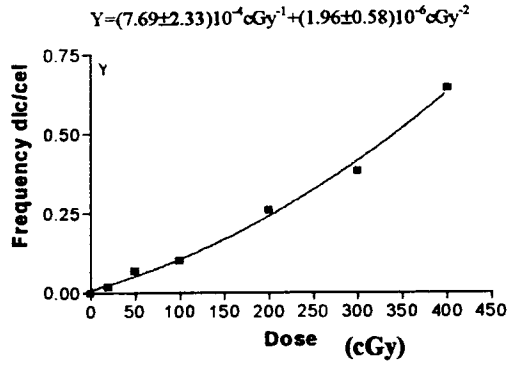

Figure 4. Frequency of chromosomal aberrations exposed to ^{137}Cs *in vitro*

IV. CONCLUSIONS

As the interpretation of radiation absorbed dose in exposed persons utilizing calibration curve of others laboratories can bring about some incertanties in the radioexposed avaliation, the IAEA recommends that each laboratory which elaborates cytogenetic dosimetry padronizes it's own calibration curve to minimize such incertainties. There were analysed blood samples of health donors, no smokers, exposed to radiation of ^{60}Co and ^{137}Cs with the dose rate of 5 cGy.min.$^{-1}$ and it was observed that every donors presented similar results of the frequency of chromosomic aberrations. The difference observed when compared with others donors and literature data may be explained by interindividual differences donors had or in the cultive method.

Considering the objective of this work that is to provide the basic and methodological fundamental utilized by cytogenetic dosimetry and to contribute in this area by elaborating dose-response curve to estimate the dose of radiation exposed persons, we conclude from the obtained data of cytogenetic analyses of the two groups of two donors that radioinduced chromosomic aberration index in peripheral blood lymphocytes is higher in ^{137}Cs when compared to ^{60}Co; the frequency of chromosomic aberrations showed that as the dose elevates, there was a progressive increase in the quantity of chromosomic aberrations, as same as the distribution of aberrations per cell.

REFERENCES

(1) Lloyd, D. C. and Purrott, R. J. **Chromosome aberration analysis in radiological protection dosimetry.** Radiat. Prot. Dosim., p 1-19, 1981.

(2) INTERNATIONAL ATOMIC ENERGY AGENCY. **Biological dosimetry chromosomal aberration analysis for dose assessment**. STI/PUB/10/260, Vienna, IAEA, 1986.

(3) Bauchinger, M.; Schmid, E.; Streng, S. and Dresp, J. **Quantitative analysis of the chromosome damage at first division of human lymphocytes after ^{60}Co γ irradiation**. Radiat. Environm. Biophys., vol.22, p 225-229, 1983.

(4) Bender, M. A. and Gooch, P. C. **Types and rates of X-ray induced chromosome aberrationns in human blood irradiated in vitro.** Proc. Nat. Acad. Sci (USA), vol.48, p 522-532, 1962.

COMISSÃO NACIONAL DE ENERGIA NUCLEAR/SP IPEN