CHROMOSOMAL ABERRATIONS INDUCED BY IONIZING RADIATION AT DIFFERENT DOSES

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Abstract: The present study was designed to evaluate the *in vitro* effect of ionizing radiation in human peripheral blood mononuclear cell cultures. We studied the chromosomal aberration induced by X ray in normal human peripheral blood mononuclear cell cultures. Different doses (0.8 Gy, 2 Gy and 5 Gy) for irradiation of human cell cultures were used for this study. Chromosome aberration analysis was carried out at 48 and 72 hours from irradiation. Radiation-induced chromosomal modifications were detected by karyotyping. The obtained results strongly suggest that normal cells can repair at low doses.

INTRODUCTION

All living beings on the Earth are exposed to cosmic radiation, solar radiation, external terrestrial sources and radon. Exposure of cells to ionizing radiation may affect directly (dissociation and ionization) and indirectly (water radiolysis) the cell, especially the DNA molecule (Benjamin Ciola 1970, Morgan W. E., Sowa M. B 2005). Cell cultures are very used in radiobiology research for studying the chromosomal aberrations (Bender M.A. and Gooch P.C. 1966). Double-strand breaks represent the most lethal form of DNA damage (Marekova M., Vavrova J., Vokurkova D., Psutka J. 2003). Chromosome aberrations induced by ionizing radiation in G_0 human peripheral lymphocytes can be classified as unstable (dicentrics, centric rings and acentric fragments) or stable (various kinds of translocations) (Natarajan et al., 1992, 1994, Radha Saraswathy and A.T. Natarajan, 2000).

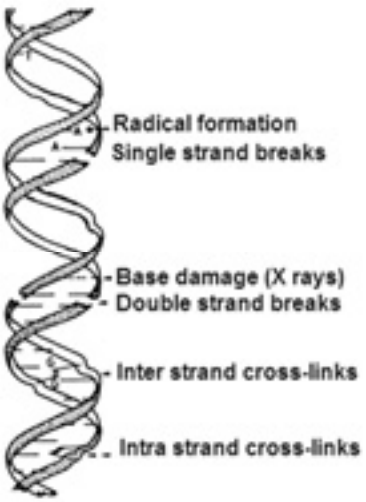

Figure 1. Radiation induced DNA damages (Technical reports series no. 405, 2001)

Unstable damages can be eliminated by mechanisms of DNA repair whereas stable aberrations persist and lead to mutations (Natarajan *et al.*, 1991; Lucas *et al.*, 1992; Boei *et al.*, 1994, Technical reports series no. 405, 2001). The

frequencies of structurally chromosomal aberrations in peripheral lymphocytes has been used since the mid 1960s for estimated absorbed radiation dose of accidentally irradiated persons (Schneider G. J., Chone B. and Blonnigen T., 1969, Technical reports series no. 405, 2001).

In the present study we proposed to estimate the cytogenetic changes induced by X ray in peripheral blood cultures. To irradiate the peripheral blood cultures, different doses (0.8 Gy, 2 Gy and 5 Gy) were used for this study.

MATERIALS AND METHODS

1. Cell culture and cell conditions. Peripheral blood samples were collected in sodium heparinised vaccutainer tubes through venipuncture from a healthy donor. The peripheral blood has been cultured in RPMI 1640 medium, supplemented with 10 % fetal bovine serum (FBS, GIBCO), antibiotics (Hy Clone), L-glutamine and heparine. To stimulate the growth of T lymphocytes, phytohemagglutinin was added. The cell cultures were incubated in an incubator at 37°C in a humidified atmosphere of 5% CO2. For 10 mL of complete medium, 0.7 mL whole blood was added.

2. X ray irradiation. Irradiation was performed at room temperature using a X-ray SRT100 (Department of Radiology in "St. Spiridon" Hospital, Iasi) at a dose rate of 2.27 Gy/min (STR100, Topex, Inc, 10 Precision Drive Danbury). Whole blood cultures used in this study were irradiated at 24 hours after the culturing and different doses, 0 Gy, 0.8 Gy, 2.5 Gy and 5 Gy, were used. After irradiation, culture flasks were placed back in the incubator (37°C).

3. Cytogenetic analysis. Karyotyping is a technique to examine cell chromosomes, which can identify the chromosomal abnormalities. Mitotic chromosome analysis was performed on peripheral blood cultures (Charles D. Bangs, Timothy A. Donlon, 2005). The technique used was optimized in the Laboratory of Immunology and Genetics Hospital "St. Spiridon" Iasi. The cytogenetic changes were studied at 48 hours and 72 hours after irradiation.

The first step in cytogenetic analysis was blocking the cell cycle. Colcemid (25 µL colcemid for 4.5 mL medium) was added in flasks with whole blood cultures, for 50 minutes, at 72 hours after culturing and 96 hours, respectively. The cells from whole blood culture were treated with KCl (75 mM) hypotonic solution for 16 min at 37°C and fixed in methanol-acetic acid (3:1) at 4°C. For first fixation, the sample has been kept in a refrigerator for 30 minutes, 20 minutes for two fixation and 10 minutes for last fixation. The fixed cells were dropped onto wet slides and stained with Giemsa.

4. Chromosome banding. Chromosomes were banded to facilitate the identification of chromosomal aberrations. The technique used was optimized in the Laboratory of Immunology and Genetics Hospital "St. Spiridon" Iasi. The first step in chromosome banding was maintaining the slides at 80°C for 50 minutes. Slides were placed in SSC 2X (the saline-sodium citrate buffer) at 60°C, for 20 minutes. Then followed the tripsinization. The slides were washed in three solutions (Trypsin solution, pH = 7.1, Hanks, pH = 7 and Sorensen phosphate buffer). The staining of slides with PBS (Phosphate buffered saline) solution and Wright solution (3:1). The slides were viewed at Nikon E600 microscope and for caryotyping technique was used the Genetix CytoVision system.

RESULTS AND DISCUSSIONS

We irradiated normal whole blood cultures with different doses 0 Gy, 0.8 Gy, 2.5 Gy and 5 Gy. The chromosomal aberrations induced by X radiation were analyzed using the cytogenetic technique at 48 and 72 hours from irradiation.

Cytogenetic analysis was performed by standard karyotype and included: initiation of lymphocyte cultures, chromosome preparation and chromosome banding. For each case 30 metaphases were examined. The normal human male karyotype is shown in figure 2.

Figure 2. The normal human male karyotype 46, XY (irradiated with 0 Gy).

1. Dose 0.8 Gy. Chromosomal abnormalities appeared after low doses of radiation. Analysing slides from cell cultures irradiated with 0.8 Gy, only one normal metaphase was found at 48 hours after irradiation, and two normal metaphases at 72 hours from irradiation. In figure 3 was represented an abnormal metaphase due to 0.8 Gy irradiated sample.

Figure 3. Abnormal metaphase. Kariotype of irradiated sample with 0.8 Gy.

2. Doses 2.5 and 5 Gy. At 2.5 Gy and 5 Gy we did not found any normal metaphases. In figure 4 was represented two abnormal metaphases due 2.5 Gy and 5 Gy irradiated samples.

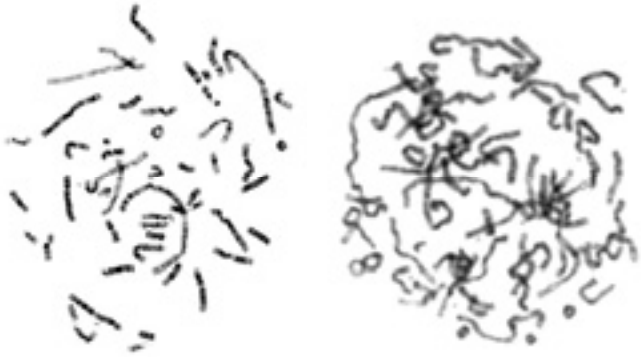

Figure 4. Abnormal metaphases. Karyotype of irradiated samples with 2.5 Gy (left) and 5 Gy (right).

Number of abnormal metaphases decreased progressively with time. The only stable type aberrations persist for long time, many of damages were repaired immediately (Technical reports series no. 405, 2001). In figure 5 was represented the number of abnormal metaphases according to dose. The number of abnormal metaphases increased with increasing radiation dose, and in the samples irradiated with 5 Gy most of the cells were dead, while those still alive were abnormal.

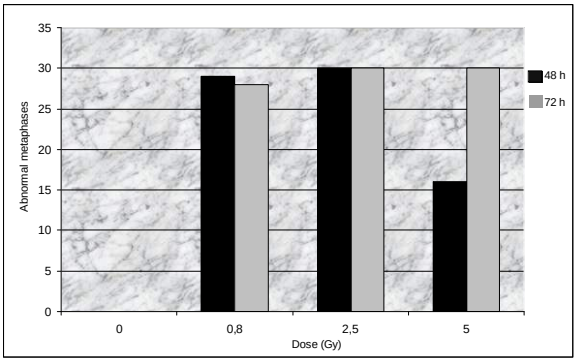

Figure 5. Graphic representation of abnormal metaphases for doses used, analyzed after 48 and 72 hours from irradiation.

In figure 6 was represented the number of normal metaphases according to dose. For samples irradiated with 0.8 Gy the number of abnormal metaphases decreased with time, but for samples irradiated with 2.5 Gy and 5 Gy, the number of abnormal metaphases increased with time.

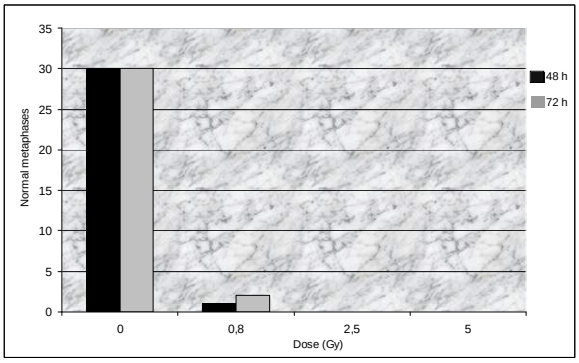

Figure 6. Graphic representation of normal metaphases for doses used, analyzed after 48 and 72 hours from irradiation.

Also we were determined the mitotic index as a measure of cell proliferation. For mitotic index calculation we used the next express:

Mitotic index = Number of metaphases/1000 nuclei

Figure 7 shows that at 72 hours after irradiation the mitotic index increased for all samples, because the cultures were croped to a mitogen (phytohemagglutinin) for stimulated T lymphocytes in culture. The first person who showed that peripheral human leukocytes can be stimulated by phytohaemagglutinin (PHA) to undergo in vitro mitoses, was Nowell (Technical reports series no. 405, 2001, Nowell P.C.,1960).

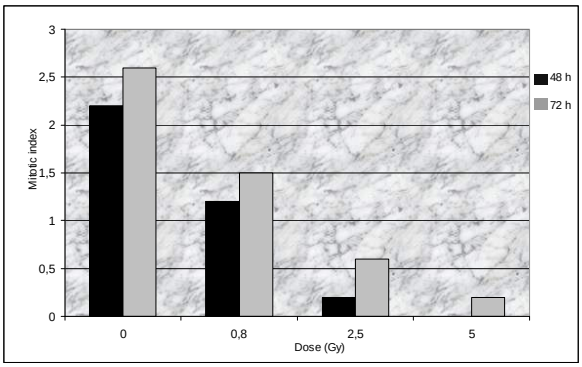

Figure 7. Mitotic index variation depending on the dose of irradiation.

Analysing the karyotype of irradiated sample with 0.8 Gy, we noticed there were 46, XY chromosomes and a ring chromosome. For sample irradiated with 2.5 Gy, the metaphase had more chromosome than a normal metaphase. We also noticed two ring chromosomes in metaphase analysed. For the sample irradiated with 5 Gy, the metaphase was abnormal with polyploidy and 4 ring chromosomes.

Our study confirms the results obtained from literature and we also indentified structure abnormalities radio-induced: ring chromosomes, markers, translocations, deletions (Technical reports series no. 405, 2001, Natarajan A.T., Obe G., 1978, Darroudi F., Natarajan A.T., Vander Schans G.P., Vanloon A.A.W.M., 1989).

This experiment is a proper model for the study of structural changes of cells under the influence of various doses of radiation from different intervals of time.

Ionizing radiations used in medicine are of great importance, most research in radiology being designed to study dose-response relationship for determination the effect of radiation dose planned or accidental.

CONCLUSIONS

The cytogenetic study revealed that only at low dose of ionizing radiation (0.8 Gy) normal metaphases could still be found, in both cultures (48 and 72 hours). The cells irradiated with higher doses of ionizing radiations (2.5 Gy and 5 Gy) showed no normal metaphases in none of the above mentioned cultures. Mitotic index was found to be significantly decreased in a dose dependent manner.

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