ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences
(ISSN: 0975-8585)

RESEARCH ARTICLE

Radiation-induced alterations in hematopoietic system of Swiss albino mice following exposure of low to moderate doses of gamma radiation.

Savita Verma*.

Division of Molecular and Radiation Biosciences, Institute of Nuclear Medicine and Allied Sciences (INMAS), DRDO, Brig. S.K. Mazumdar Marg, Delhi-110054, India.

ABSTRACT

Ionizing radiation can have severe lethal effects depending upon the dose and the organs exposed. With the exposure of low to moderate dose of radiation it is difficult to predict the damage until unless it becomes alarmed. The study aimed to discuss the effect of low to moderate dose ionizing radiation on some blood components and cytogenetic damage in mice. Variation in the blood parameters was studied at the doses of 0.1Gy, 0.2Gy, 0.5Gy, 1Gy, and 2Gy. Evaluation of cytogenetic damage was carried out at doses 0.5Gy to 2Gy. The animals were dissected at different time intervals. Significant reduction in total blood leucocytes counts was observed in dose dependant manner. No significant variation in red blood cells (RBCs) count, hemoglobin (Hb) and platelets was observed at all the studied doses of radiation. Significant increase in the number of micronucleated cells, dicentrics and acentric fragments in bone marrow cells of mice was observed in response to radiation dose. The present findings suggest that radiation causes significant reduction in leucocytes counts and induces cytogenetic damage in dose dependant manner. Much effort is needed for establishment of protocols for medical management in case of radiation accidents and therapeutic exposure.

Keywords: Ionizing radiation, Cytogenetic, Micronuclei, Chromosomes

https://doi.org/10.33887/rjpbcs/2021.12.5.6

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Eminent use of the nuclear energy in diversified field for human applications increases the chance of exposure to ionizing radiations. Detonation of the atomic bombs, nuclear reactor accidents and terrorist activities result in large-scale exposure to radiation [1]. More over radiotherapy, used in treatment of certain diseases, is also found to be associated with certain acute side effects of radiation such as bone marrow suppression, damage to epithelial surfaces like skin, oral, pharyngeal and bowel mucosa, infertility and cancer [2]. The degree of appearance of radiation syndrome depends on the organ's sensitivity towards radiation, type of radiation, dose, fractionation, and the individual's sensitivity towards radiation. Although, many of these side effects are reversible in nature, but the literature data strongly supports the probability of transmissible genetic deviations after exposure to ionizing radiation resulting in carcinogenic effects [3]. Possibility of increase in cancer due to diagnostic x-rays, breast carcinoma, primary malignancies, lung carcinoma in women undergoing radiotherapy is increasing day by day in developing countries [4]. Side-effects produced by radiotherapy occur because during fractionated radiotherapy, healthy tissues that surrounding the tumor area are also exposed to low doses of ionizing radiation. Therefore, the extent of genotoxicity and biological effects of these low doses of ionizing radiation on the healthy tissues is needed to be determined. Prompt recognition and treatment can prevent serious complications.

The hematopoietic system is highly radiosensitive due to rapid proliferation rate and suppression of hematopoietic system has been considered as the most life-threatening consequences following radiation exposure [5]. Ionizing radiation damages the hematopoietic stem cells, progenitor cells, mature cells as well as hematopoietic microenvironment, leading to myelosuppression or bone marrow failure [5]. Irradiation can induce damage to the cellular macromolecules, either directly or by the production of free radicals. Radiation-induced damage to the DNA appears in the form of chromosomal aberrations, are highly quantifiable manifestations of radiation-induced DNA damage [6]. Quantification of radiation-induced chromosomal aberration for estimation of absorbed dose can help in medical management including triage [7]. In the present study we attempted to find the extent of damage induced by radiation and the post irradiation period required for recovery to the haemotopoietic system at different (low to moderate) doses of ionizing radiations. The study was performed on mice at a dose range of 0.1Gy to 2Gy at the post irradiation intervals ranging from 24hrs to 40th day.

MATERIALS AND METHODS

Animals and γ-ray irradiation

Experiments were performed on pathogen free inbred 2-4 months old, Swiss albino mice (24$\pm$2 gm). Animals were housed in polypropylene cages bedded with sterilized rice husk under the 12h cycles of light and dark and fed with standard food pellet and water *ad libitum*. Mice were exposed in ^{60}Co gamma chamber at the dose rate of 0.36 Gy/min. Fresh air was circulated continuously in the irradiation chamber to avoid hypoxic conditions. Dosimetry was carried out using Baldwin Farmer's secondary dosimeter and Fricke's chemical dosimetry method. Institutional Animal Ethics Committee (IAEC) guidelines were strictly followed for conducting the experiments.

Experimental protocol

Animals were divided in control and irradiated groups having 6 animals in each group. Control animals were sham irradiated. Mice were exposed to different doses of gamma radiation (0.1Gy, 0.2Gy, 0.5Gy, 1Gy, 2Gy). All the experimental animals were observed for symptomatic and body weight changes. Mice were anesthetized and sacrificed at various time intervals (24hrs, 48hrs, 72hrs, 5th day, 10th day, 15th day, 25th day, 40th day) for hematological studies. Blood was drawn by heart puncture of animals. Cytogenetic studies were carried out at 0.5Gy, 1Gy, 2Gy doses at 24hrs, 48hrs and72hrs after radiation exposure.

Hematology

Irradiated and control animals were sacrificed at different time intervals. Blood was collected in the syringe having EDTA, by heart puncture. Red blood cells (RBC), hemoglobin and platelets were

measured by automated cell counter (SYSMEX K- 4500, Japan). Total leucocytes count was estimated by diluting heparinized blood with Turk's fluid for the lysis of the cells other than leucocytes. The diluted sample was loaded in Neubauer's chamber and cells were counted in all the four chambers under light microscope (Olympus BX-50).

Micronucleus (MN) Assay

Micronuclei in mice bone marrow cells were scored by the method described earlier [8]. After dissecting, both the femora were removed and marrow cells of one femur were used for micronuclei and from other were processed for chromosomal analysis. For micronuclei, the bone marrow cells were aspirated in PBS with the help of syringe. To the centrifuged suspension, few drops of fetal bovine serum were added and smear was drawn on clean slides. The slides, after fixing in methanol, were stained for 10 min with May-Grunwald Giemsa stain diluted in Sorensen's buffer (pH 6.8). The slides mounted in DPX were observed under microscope. For each animal, 500 cells were scored and percentage of micronucleated cells was calculated.

Preparation of metaphase plates

The animals were injected with colchicine (5mg/kg/b.wt.) intraperitoneally, 2hrs before scarifying. Metaphase plates were prepared from bone marrow cells as the method described earlier [9]. Briefly, the marrow cells were flushed in hypotonic and incubated at 37^{o}C for 30 min. After centrifugation, the cells were fixed in Carnoy's fixative and slides were prepared by air dry method. Slides stained in 5% Giemsa were scored for dicentric and acentric fragments. A total of 50 metaphase plates were counted for each group.

Statistical analysis

Data was presented as mean±standard error of mean (SEM) and statistical analysis was performed using analysis of variance (ANOVA) to test the significant difference between the groups. Significance levels were set at $p<0.05$, $p< 0.01$ and $p<0.001$.

RESULTS

Symptomatic observation and body weight changes

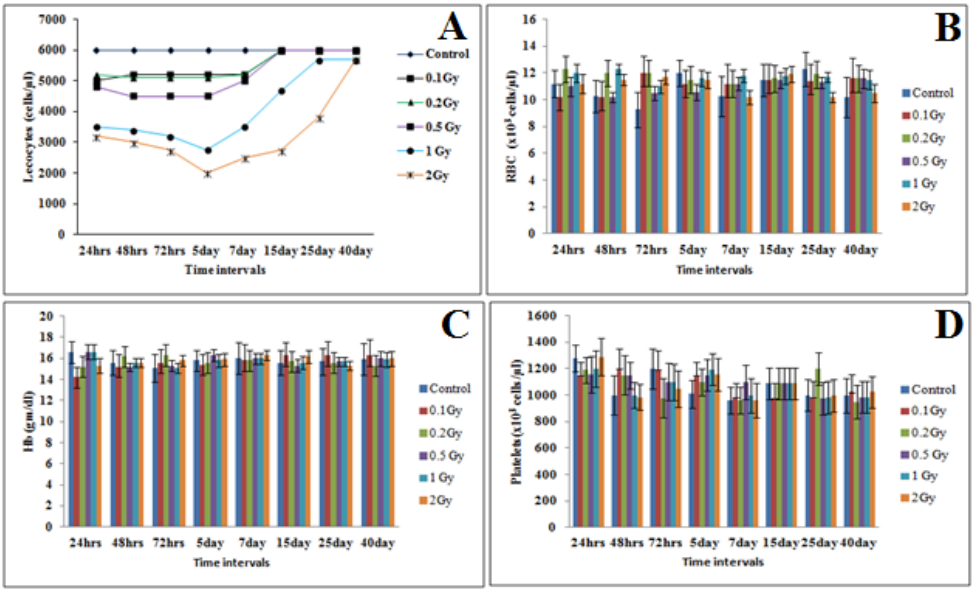
At all the observed doses of radiation no apparent sign of radiation poisoning was observed. In the normal course body weight increased smoothly. Animals exposed to 0.1Gy to 0.5Gy followed the similar weight gain pattern as controls at all the time intervals. 1Gy dose exposed animals showed stable weight till 5th day and then adopted the normal path of weight progression. At 2Gy marginal fall (non significant) in weight was observed after 48 hrs and continued till 15th day, thereafter recovery started (data not shown).

Effect of gamma radiation on hematological changes

It was observed that total leucocytes count goes in linearity with the dose exposure (Figure 1A). At 0.1-0.5 Gy, about 16-20% fall in TLC was observed at 24hrs after radiation exposure as compared to control group ($p<0.05$) while this fall was 41-46% at 1Gy and 2Gy doses, which was significantly higher ($p<0.01$) than the control values. The reduction in counts appeared to sustain for 5 days and at 1-2Gy doses and magnitude of the fall was comparatively high from 48hrs to 5th day, thereafter recovery started. TLC approached to normal after 15th day for 0.1-0.5 Gy doses and 40th day for 1-2Gy radiation doses. No significant reduction in RBC counts, hemoglobin and platelets was observed at all the studied doses of radiation (Figure 1B-D).

ISSN: 0975-8585

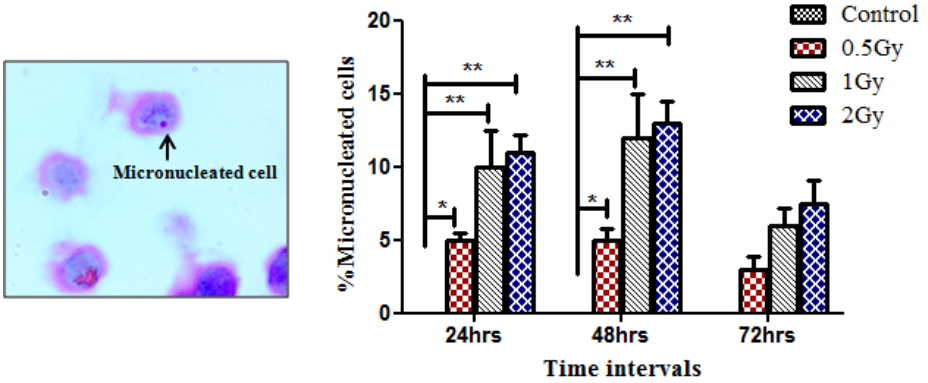
Figure 1: Effect of different doses of gamma radiation on hematological alterations in mice at different time intervals. (A) Leucocytes count (B) RBCs count (C) Hemoglobin (D) Platelets count. Error bars are SEM for n = 6.



Effect of gamma radiation on micronuclei formation

Bone marrow micronucleated cells in all the treated and control mice were counted at 24hrs, 48hrs and 72hrs after radiation exposure (0.5Gy, 1Gy and 2Gy). A dose dependant elevation in the number of micronucleated cells was observed at all the studied doses (Figure 2). At 1-2Gy dose, number of micronucleated cells enhanced significantly (10±2%, p<0.001) after 24hrs and were maximum at 48hrs (13±2%, p<0.001) when compared to control. The numbers of micronucleated cells diminished at 72hrs after exposure of gamma irradiation.

Figure 2: Effect of different doses of gamma radiation on formation of micronucleated cells in bone marrow of mice. Photomicrograph represents micronucleated cell in bone marrow of irradiated mice. Bar graph shows percentage of micronucleated cells at different doses of radiation. Error bars are SEM for n =6. *p< 0.01, **p< 0.001.



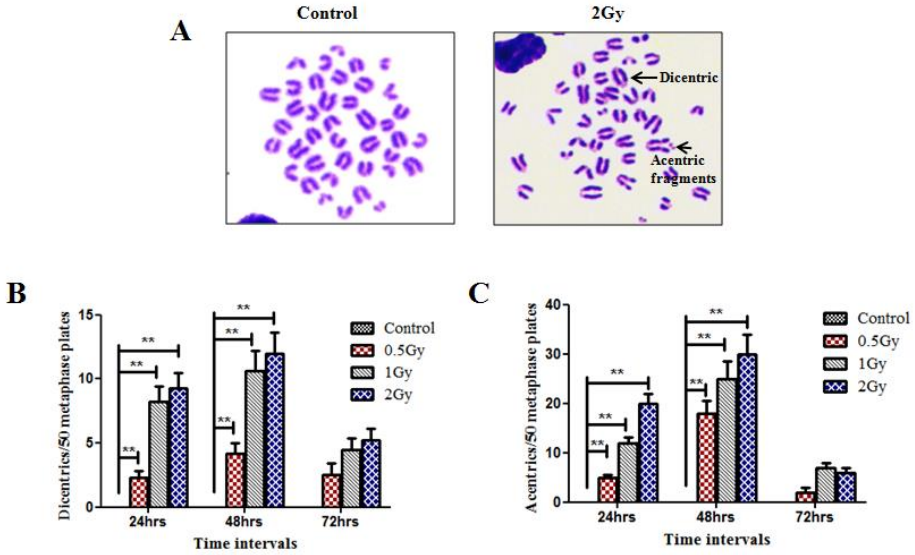
Effect of gamma radiation on chromosomal aberrations

Dicentrics and acentric fragments were monitored in the metaphase plates at different doses of radiation after 24hrs, 48hrs and 72hrs time intervals. A dose dependant elevation was found in the frequency of dicentrics and acentric fragments (Figure 3A-C). Dicentrics increased significantly after 24hrs at all the doses (0.5Gy: 2.3±0.5, p<0.05, 1Gy: 8.25±2.2, p<0.001, 2Gy: 9.3±3.5, p<0.001, all groups control vs. radiation) and were maximum at 48hrs (0.5Gy: 4.2±1.5, p<0.05, 1Gy: 10.6±2.5, p<0.001, 2Gy: 12.5±3.1, p<0.01; all groups control vs. radiation) and declined thereafter (Figure 3B). The frequency of getting the acentric fragments was similar to dicentrics (Figure 3C). Although, a sharp increase was

ISSN: 0975-8585

observed in the number of acentric fragments after 48hrs (0.5Gy: 18.2±3.5, $p<0.05$, 1Gy: 25.0±4.5, $p<0.01$, 2Gy: 32.0±3.6, $p<0.01$), maximum numbers of dicentrics and acentric fragments were found at 2Gy dose.

Figure 3: Effect of different doses of gamma radiation on cytogenetic damage in mice bone marrow cells. (A) Representative photomicrographs of metaphase plates of control and 2Gy irradiated mice. (B) Bar graph represents dicentrics at different dose of radiation. (C) Bar graph represents acentric fragments in bone marrow cells of mice at different doses of radiation. Error bars are SEM for n =6. **p< 0.001.



DISCUSSION

The study notes that continuous dose dependant cascades of changes are initiated in the hematopoietic system of mice by the exposure of ionizing radiation. Of the findings observed herein, the decrease in leucocytes is directly related to the radiation dose. Although at very low doses 0.1-0.5Gy no significant fall in WBC was observed but at 1-2Gy decline in WBC was found to be directly related to the radiation dose as reported in earlier studies [10]. The length of the latency period between the radiation exposure and decrease in blood cell numbers depends on the degree of damage and on the normal lifetime of that particular class of blood cells. Radiation-induced mortality at higher doses results from hemorrhage and hematopoietic suppression [11]. In the current study, decline in RBC parameters like erythrocyte counts, hemoglobin and platelets was not recorded at all the observed doses. Since the life span of red cells is more as compared to white cells, so they replaced more gradually, and the effect is seen somewhat later. The blood-forming cells, in the bone marrow, are highly susceptible to radiation injury. The circulating mature white blood cells and platelets are extremely radiosensitive while red blood cells are relatively radioresistant [12]. All the blood cells are constantly being replaced by new ones, but still leucocytes, having short life span in circulating blood, need to be reconstituted because they are rapidly reduced after exposure to radiation. If replacements are not forthcoming because of damage to the bone marrow, the population of white blood cells will drop remarkably after exposure [13]. In our study, a dose dependant decline in total leucocytes count was observed even at 24h after radiation exposure however, the animals recovered because magnitude of the damage to progenitor stem cell of bone marrow is not so extensive, they repopulate and produce the required blood cells.

Biological effects of radiation, whether it is cytotoxicity, mutation or malignant transformation, would occur as a result of DNA damage in the target cell [14]. Radiation induces transmissible genetic instability in cells that enhances the rate of malignancy [15]. Scoring of micronuclei and chromosomal aberrations provide direct assessment of exposure of ionizing radiation. Micronuclei also serve as predictor of carcinogenic risks by many researchers [16]. Micronuclei are acentric fragments or whole chromosomes formed by failure to incorporate into the daughter nuclei during mitosis and remained encapsulated in the either of the cell [17]. In our study a dose dependant response of micronucleated cells was observed. The frequency of formation of micronuclei is in accordance with the earlier findings [18,19]. The number of micronucleated cells was less at 24hrs after radiation because they appeared at the end of first mitotic division after radiation. An enhanced frequency of micronuclei was found at 48h

after radiation at all the observed doses. Thereafter the number of micronucleated cells declined which could be attributed to loss of aberrant cells from cell cycle and delaying effects of radiation.

Unstable-type aberrations such as dicentrics, rings and fragments are likely to be suitable markers for detecting chromosome instability after exposure to ionizing radiation [20]. The frequency of dicentric chromosomes forms the basis for cytogenetic radiation dosimetry. Our study showed dose dependant elevation in the occurrence of dicentrics at the dose range of 0.1Gy-2Gy. Dicentrics were substantially higher at 2Gy radiation exposure which declined at later time intervals. There appear to be a close correlation between the occurrence of dicentrics and acentric fragments. The frequency of obtaining the dicentrics and fragments is in accordance with the previous studies [21]. The cells carrying unstable kinds of aberrations do not persist through the next cell cycle and are eliminated by apoptosis [22].

The findings from the current study revealed that the hematopoietic markers can be used to assess the radiation dose absorbed in the body. At the time of the radiation accident, it is essential to measure the dose received by exposed population. In the current study we found a strong correlation between the dose absorbed and changes in the blood and cytogenetic parameters. Assessment of radiation doses can help in optimizing and individualizing subsequent medical management in radiation accidents scenarios.

ACKNOWLEDGEMENTS

Author thanks to Director, Institute of Nuclear Medicine & Allied Sciences, Delhi for his administrative support.

REFERENCES

[1] Little M P. Br Med Bull 2003; 68 (1):259-275.
[2] Majeed H and Gupta V. Adverse effects of radiation therapy. StatPearls 2020[Internet].
[3] Desouky O, Ding N, Zhoub G. J Radia. Res Appl Sci 2015; 8(2): 247–254.
[4] Iranmakani S, Mortezazadeh T, Sajadian F, Ghaziani M F, Ghafari A, Khezerloo D, Musa A E. Egypt. J Radiol Nucl Med 2020; 51(1):1-22.
[5] Lu Y, Hu M, Zhang Z, Qi Y, Wang J. Radiation Medicine and Protection 2020; 1(1):31-34.
[6] Huang L, Snyder A R, Morgan W F. Oncogene 2003; 22 (37):5848-5854.
[7] Rana S, Kumar R, Sultana S, Sharma R K. J Pharm Bioallied Sci 2010; 2(3):189.
[8] Chaubey R C, Bhilwade H N, Joshi B N, Chauhan P S. Int J Radiat Biol 1993; 63(2):239–245.
[9] Yosida T H and Amano K. Chromosoma 1965; 16(6):628 – 667.
[10] Billings PC, Romero-Weaver AL, Kennedy AR. American Society for Gravitational and Space Research. 2014; 2(1):25.
[11] Thrall KD, Lovaglio J, Murphy MK, Cataneo RN, Chaturvedi A, Mundada M, Patel U, Phillips M. Health Phys 2013; 105(3):245-252.
[12] Hosseinimehr SJ. Drug Discovery Today 2007; 12(19-20):794-805.
[13] Walker RI, Conklin JJ. Military Radiobiol 1987; 1-8.
[14] Brenner DJ, Doll R, Goodhead DT, Hall EJ, Land CE, Little JB, Lubin JH, Preston DL, Preston RJ, Puskin JS, Ron E. Proc Natl Acad Sci 2003;100(24):13761-13766.
[15] Lorimore SA, Coates PJ, Wright EG. Oncogene 2003; 22(45):7058-7069.
[16] Durante M and Formenti SC. Front. Oncol 2018;8:192.
[17] Fenech M, Kirsch-Volders M, Natarajan AT, Surralles J, Crott JW, Parry J, Norppa H, Eastmond DA, Tucker JD, Thomas P. Mutagenesis 2011; 26(1):125-132.
[18] Jagetia GC and Aruna R. Mutat Res Genet Toxico. Environ Mutagen 1997; 393(1-2):157-163.
[19] Jindal A, Nunia V, Goyal PK. Indian J Nucl Med 2006; 21(1):12.
[20] Baudin C, Bernier MO, Klokov D, Andreassi MG. International journal of molecular sciences. 2021 Jan; 22(14):7504.
[21] Jagetia GC, Venkatesha VA, Reddy TK. Mutagenesis 2003;18(4):337-343.
[22] Dawar S, Lim Y, Puccini J, White M, Thomas P, Bouchier-Hayes L, Green DR, Dorstyn L, Kumar S. Oncogene 2017; 36(19):2704-2714.