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EFFECT OF ELECTRON BEAM RADIATION ON HEMATOPOIETIC CELLS OF SWISS ALBINO MICE

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Abstract:
Ionizing radiation which results in the free radical formation and it leads to damage of biological macromolecules such as DNA, proteins, lipids. 36 male Swiss albino mice were used for survival assay, to find out the lethal dose of Electron Beam Radiation. It was found to be 10Gy was the lethal dose for mice. Different dosages (4Gy, 6Gy and 8Gy) of electron beam radiation were used to study the micronucleus formation in irradiated mice. The results showed micronucleus formation will increase linearly with radiation dosage.
Keywords : Electron beam radiation, LD_{50}, Micronucleus

Running title: Micronucleus Induction by Sub Lethal Ionizing Radiation

Introduction

Radiation therapy has been used in cancer treatment for many decades; it is used to eradicate cancer and as a palliative to relieve pain associated with metastases. In the course of treatment, radiation produces numerous biological perturbations in cells; because normal cell toxicity limits the doses used in effective treatment, approaches are designed to strike a balance between eliminating cancer cells and protecting normal tissues. The primary focus in radiotherapy is to increase DNA damage in tumor cells, as double strand breaks are important in cell death. Another course of action is to alter cellular homeostasis, modifying signal transduction pathways, redox state, and disposition to apoptosis. The cellular changes ideally would enhance the killing of tumor cells while reducing the probability of normal cell death [1, 2, 3].

Ionizing radiation consists of electromagnetic radiation (photons), including X-rays and gamma rays, and particulate radiation, such as electrons, protons, and neutrons. Clinical radiation oncology uses electromagnetic radiation and particulate radiation, mostly electrons and to a lesser extent neutrons and protons [4]. Radiation damages cells by direct ionization of DNA and other cellular targets and by indirect effect through ROS [5].

Radiation induces breaks in the chromosomes and chromatids. This break leads to chromosomal aberrations and micronucleus formation. Micronuclei induced by sub lethal ionizing radiation have been studied in mouse bone marrow cells and peripheral blood erythrocytes. In humans they are scored in the mitogen stimulated peripheral lymphocytes *in vitro* [6].

Electron beam radiation is a form of ionizing energy that is generally characterized by its low penetration and high dosage rates. It is a concentrated, highly charged stream of electrons, generated by particle accelerators which are capable of producing beams that are either pulsed or continuous. This high energy electrons are used for various purposes in the field of biology, it it is also used in the radiation therapy [7].

With this background our aim of the study is to evaluate the lethal dose of electron beam radiation on Swiss albino mice. Also to score the sub lethal whole body electron beam radiation induced micronucleus in bone

marrow cells of mice.

MATERIALS AND METHODS

Animal care and handling

Animal care and handling was carried out according to the guidelines set by WHO (World Health Organization; Geneva, Switzerland). The institutional animal ethical committee has approved this study. Swiss albino mice aged 6 -8 weeks and weighing 25±5 g, taken from an inbred colony, was used for this study. The mice were maintained under controlled conditions of temperature and light (light: 10 h; dark: 14 h). Four animals were housed in a polypropylene cage containing sterile paddy husk (procured locally) as bedding throughout the experiment. They were provided standard mouse feed and water ad libitum.

Survival assay

36 male Swiss albino mice were used for the Survival assay. These animals were divided into 6 groups. Each group contains 6 animals each. These animals were irradiated to 4Gy, 6Gy, 8Gy, 10Gy, 12Gy and 14Gy radiation dosages. The percentage of mice surviving 30 days after exposure against each dose will be used to construct survival dose response curve [8].

Irradiation

The irradiation work was carried out at Microtron centre, Mangalore University, Mangalore, Karnataka, India. The animals were restrained in well-ventilated perspex boxes and exposed to whole-body electron beam at a distance of 30 cm from the beam exit point of the Microtron accelerator at a dose rate of 72 Gy/min.

Micronucleus assay

The mouse bone marrow micronucleus test was carried out according to the method described by Schmidt [9] by evaluation of chromosomal damage in experimental animals. The animals exposed to sub lethal dose 4Gy, 6Gy and 8Gy electron beam radiation were scarified on 31st day post irradiation. The bone marrow cells from femur were flushed in the form of a suspension into a centrifuge tube containing 5% BSA. The cells were dispersed by gentle pipetting and collected by centrifuge at 2000 rpm for 5 min at 4°C. The cell pellet was resuspended in a drop of BSA and bone marrow smear were prepared. After air drying the smear were stained with May-Grunwald/Giemsa. Micronucleated polychromatic erythrocytes and Non chromatic erythrocytes were observed under Microscope. The percentage of micronucleated polychromatic erythrocytes (MnPCEs), micronucleated normochromatic erythrocytes (MnNCEs) and ratio of PCE to (PCE + NCE) was calculated.

Statistical analysis:

All values were expressed as Mean ± SD. Comparison between different groups were performed by analysis of variance (ANOVA) with Bonferroni. In all these test criterion for statistical significance was $P<0.05$.

Results

The radiation dose was determined by exposing the mice with various doses (4Gy, 6Gy, 8Gy, 10Gy, 12Gy and 14Gy) of electron beam radiation. It was found to be non toxic up to a dose of 6Gy, where no radiation induced mortality was observed. A further increase in the electron beam dose to 8Gy resulted in 33% mortality. An increase in radiation dose to 10Gy caused a 50% reduction in the survival of mice. 100% of the mice died when the electron beam dose was increased to 12Gy and 14 Gy. The LD_{50} of electron beam for acute radiation induced mortality was 10Gy (Graph 1).

The whole body electron beam exposed mice showed the formation of micronucleus in the bone marrow cells. The frequency of micronuclei was increased with increase in radiation dose (Table 1).

Discussion

A single whole-body exposure of mammals to ionizing radiation results in a complex set of syndromes whose onset, nature and severity are a function of both total radiation dose and radiation quality. At the cellular level, ionizing radiation can induce oxidative stress [10] which results in the damage of biologically important

macromolecules such as DNA, proteins, lipids and carbohydrates in various organs [11, 12]. Irradiation produces different types of lesions in DNA molecule which lead the formation of micronucleus and breaks [6].

The LD_{50} of ionizing radiation such as X ray and gamma rays for mice found to be 6.5-9.5Gy (6). The present study showed 10Gy is the LD50 for mice; this might be due to the low penetrating power of electrons.

The survival assay results revealed that the death of animals in 12 and 14 Gy irradiated mice were observed after 10th day of irradiation. Death between 11th day to 30th day post irradiation is due to haemopoietic damage inflicted by radiation [13]. The result obtained by micronucleus assay justifies the haemopoietic damage.

Ionizing radiation induces micronucleus formation and DNA damage [14]. The micronucleus formation was found to be in dose dependent manner, micronucleus was found to increase linearly with radiation dose ($P<0.05$). These sub lethal dose induced damage to haemopoietic organs of mice will result in leucopenia, thrombocytopenia and haemostatic disorders [6].

Conclusions

The sub lethal doses of radiation also induce micronucleus formation in mice. The similar kind of mechanism may occur in humans during exposure of therapeutic radiation doses in radiotherapy. This damage is due to the direct radiation effect or by the effect of free radicals on cellular system. This damage can be reduced by antioxidant supplementation prior to the radiation exposure.

GRAPH 1: SURVIVAL ASSAY FOR ELECTRON BEAM RADIATION

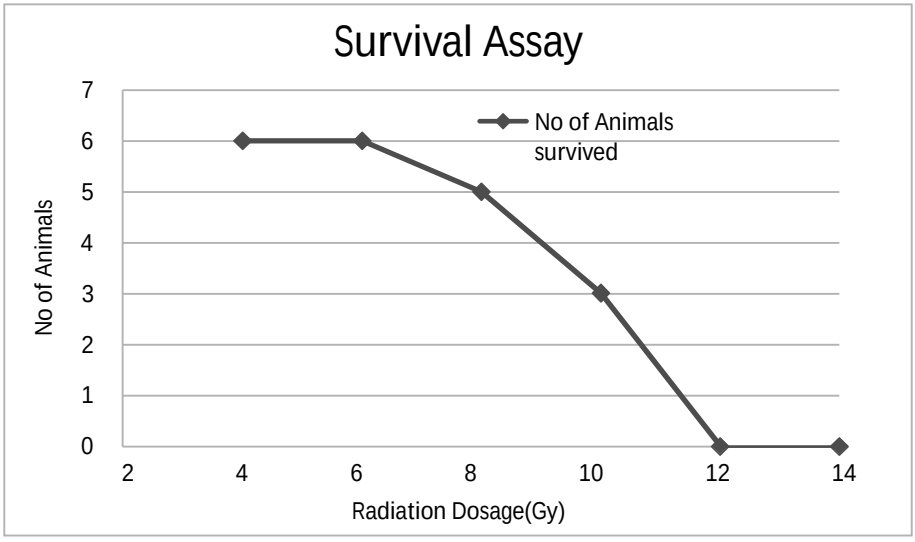

TABLE 1: EFFECT OF ELECTRON BEAM RADIATION ON MICRONUCLEUS FORMATION IN BONE MARROW CELLS OF SWISS ALBINO MICE

	4Gy	6Gy	8Gy
MnPCE/PCE (%)	12.07±0.09	28.40±4.44	32.2±0.98*
MnNCE/NCE (%)	4.08±0.10	12.67±7.30	18.47±0.44*
PCE/PCE+NCE (%)	33.03±2.35	26.50±8.32	14.43±3.16*

*P<0.05

PCE: Polychromatic erythrocytes, NCE: Non chromatic erythrocytes, MnPCE: Micronucleated Polychromatic erythrocytes, MnNCE: Micronucleated Non chromatic erythrocytes.

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