5.3 HIGH LET RADIATION BIOLOGY

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The experiments conducted in this field involved cell inactivation and chromosome aberration due to charged particle interaction with V79 and M5 cells by users from Presidency College, Kolkata using 100 MeV Oxygen and 50 MeV Lithium beam. The study is concluded and a Thesis is under preparation. There is also an ongoing research project on the germination properties, biochemical properties etc. on ion beam irradiated mustard seeds by users from MDU Rohtak, which utilised 50 MeV Li beam. User from North Eastern Hill University has also utilised 100 MeV Oxygen beam for a study on the role of Glutathion on the extent of chromosome aberration in V79 cells.

Apart from users from biology, the facility has also been used by ISRO for irradiation of electronic devices by proton beam.

5.3.1 The modulation of endogenous Glutathione and its effect on V79 cells exposed to heavy ion ^{12}C beam

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Endogenous thiols, especially the tripeptide GSH (γ- glutamyl-cysteinyl-glycin) play an important role in the protection of cells against the damaging effect of Ionizing Radiation of low Linear Energy Transfer (LET) [1,2] and Chemicals [3,6]. Treatment of cells with Buthionine Sulfoximine (BSO) sensitizes the cells to γ-ray induced chromosome aberrations and this is attributed due to failure of scavenging free radicals along with low repair efficiency [2]. It has been shown that exogenous addition of GSH could effectively reduce chromosome aberration produced by radiation such as X-rays in different systems [7] including the muntjac lymphocyte culture [1]. Several studies have shown that chemical agents which cause a radiation sensitivity of mammalian cells exposed to low- LET radiations are less effective in changing sensitivity with high-LET radiation (8,9).

The experiment was carried out with two main objectives:

a) To evaluate the pattern of induction of delay in cell cycle after high LET radiation after modulating the level of endogenous GSH

b) To establish the role of endogenous GSH on high LET radiation induced chromosome exchange aberration formation.

Materials and methods:

Cell Culture: Chinese hamster V79 cells were routinely cultured in 90 mm cell culture Petri plates (Tarson) in MEM (Himedia, India) supplemented with 10% Foetal calf serum (Biological Industries, Israel) and kept at 37° C in humidified atmosphere with 5% CO_2. In order to deplete GSH, BSO (Sigma, USA) was used. Cell culture was set up in the presence of 5-bromodeoxyuridine BrdU, (Sigma, USA). Colcemid (Gibco) is added to arrest the cells at metaphase.

Irradiation: Heavy ion irradiation (^{12}C) of LET 284 Kev/µm was carried out using Radiation Biology Beam Line of 15 UD pelletron at Nuclear Science Centre (NSC), New Delhi. Specially fabricated stainless steel rings are used as Petri plates of 2.5 cm diameter for sample irradiation. A polypropylene sheet of 6µm thick forms the base of the stainless steel rings on which cells are seeded 12 hrs. prior to irradiation. In order to deplete GSH, some samples were treated with 0.2 mM BSO 3 hrs. before irradiation.

Cells were irradiated with two fluences of 2.2 X 10^6 and 8.8X10^6 particles/ cm^2 which is equivalent to doses of 1 and 4 Gy respectively. One set was kept untreated while rest are irradiated with or without BSO. The Cells were irradiated under sterile condition, at atmospheric pressure and were exposed to ions through polypropylene film. Fresh medium was added after irradiation and culture was set in the presence of 10 µg/ml BrdU in order to differentiate between 1st, 2nd and subsequent metaphase. Cells were given 2hrs. Colcemid treatment and harvested at 48 hrs and 72 hrs.

Results:

Cell cycle kinetics: ^{12}C beam induced cell cycle delay in V79 cells, harvested at both 48 hrs. and 72 hrs. From the data it is found that there is a significant increase in the cell cycle delay between untreated cells and irradiated cells. However, there is no significant change in the cell cycle delay between irradiated cells and BSO treated cells as it was shown by AGT value and M1%.

Chromosome aberration: ^{12}C beam induced chromosomal aberration in V79 cells, harvested at 48 hours, showed that Deletion and Chromatid break are the most frequent type of aberration seen. These aberrations showed increase with increase in fluence. After BSO treatment there is an increase in these types of aberration. However, exchange type of aberration on the other hand showed a decrease in the frequency in the presence of BSO.

This is a preliminary study and therefore before reaching any conclusion we will have to perform more studies by fixing the cells at earlier hours.

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5.3.2 High LET radiation induced damages in mammalian cells

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Extensive work on various biological end points such as gene mutation, cellular transformation, cell death, cell cycle delay, genomic instability have already been carried out using high linear energy transfer (LET) radiations but little is known about lesions produced at the molecular level by high LET radiations and their transformation into chromosomal aberrations. In contrast to sparsely ionizing radiations which produces simple breaks, base and sugar damage, high LET radiation induced lesions are highly structurally complex such as breaks with chemically modified bases/sugars and cross-links. Among different types of lesions induced by ionizing radiation, DNA double strand breaks are mainly involved in the production of different classes of chromosomal aberrations in repair proficient cells as well as in cell killing. It is well evident that high LET particles are more efficient than sparsely ionizing radiation in inducing chromosomal aberrations, in particular complex rearrangements.

We have investigated cell inactivation by colony forming ability, chromosome aberration, apoptosis and micronuclei assay induced by different types of high LET radiations as well as by sparsely ionizing radiation (such as gamma ray). Chinese hamster lung fibroblast V79 cells and its mutant variety M5 cells were exposed to ^{16}O beam (LET= 613 KeV/μm), ^{12}C (LET= 295 KeV/μm) and ^{7}Li beam (LET = 60 KeV /μm) with fluence ranging from 10^6 to 10^7 particles/cm^2. The irradiation was carried out at the Radiation Biology beam line of Nuclear Science Center, New Delhi, which houses a 15UD 16 MV Pelletron accelerator [NEC, USA]. Different types of chromosomal aberrations were studied at three different time intervals (12, 24 and 48 hours) using Giemsa staining technique. The cells were also exposed to ^{60}Co gamma radiation with dose ranging from 1 to 10 Gy to observe the qualitative differences, if any, between the

chromosome aberrations caused by densely and sparsely ionizing radiation. Apoptosis study has been carried out by two different methods, observing nuclear fragmentation assay as detected by fluorescence microscopy and nuclear ladder formation using standard protocol. Both types of cells were harvested at four different incubation period (12, 24, 48 and 72 hours) for nuclear fragmentation assay. Micronuclei study, which is considered to be the result of various types of chromosomal or cellular damage, has been carried out by fluorescence microscopy after 20 hour of incubation.

Dose dependent increase of chromosomal aberrations was observed in all types of radiations but this increase was much more pronounced in case of ionizing radiation. When different types of aberrations were considered individually, unexpectedly high percentage of complex chromatid type of exchange was evident in heavy ion irradiated samples. The frequency of apoptosis increases with time as well as with dose and peaked at 48hr after irradiation with ^{16}O beam (LET= 613 KeV/μm) and gamma ray and then decreases. But in case of ^{12}C (LET= 295 KeV/μm) and ^{7}Li beam (LET = 60 KeV/μm) the frequency of apoptosis peaked at 24hr after irradiation. Number of micronuclei per cell increases with dose and reach to around 50% at highest dose. In every aspect M5 cells showed more radio resistant property over its counterpart V79 cells.