Adv. Radiation Biol. & Peace, pp. 19 - 26, 1999 ISSN 0256 - 971X

EFFECTS OF HIGH AND LOW LET RADIATION : A BRIEF REVIEW OF ANIMAL STUDIES *IN VIVO* AND *IN VITRO*

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Radiation is perhaps the singular important factor that triggered origin of life on this planet about four billion years ago. It is believed that eruption of enormous amounts of cosmic radiations randomly combined several atoms to form indefinite combinations of molecules. Some of these were the primordial amino acids, nucleic acids and the like with which began origin of very primitive forms of life. In course of evolution, again driven by the natural cosmic radiation, the primitive have finally led to the present complex forms of life that we see in us and all around us. Therefore, radiations have played a key role in the start of process of life and its progression to the present forms. In the present century, radiation, both natural and man-made, have been increasingly used in diagnostic and therapy modalities to mitigate human sufferings, particularly human cancer. The use of radiation in various industries and for generation of power are examples of mass industrial applications of radiations. Logically, therefore, it is quite obvious that radiation have potentials which can be harvested to benefit all of us. At the same time, only a few years after the discovery of X-rays in 1885, first cases of skin cancer were reported in persons who were repeatedly exposed to X-rays. The catastrophe of nuclear bomb explosion is another glaring example of destructive powers of radiation. These events highlight the damaging aspects of radiation. Thus, it is apparent that while radiation were causes of origin and maintenance of life and can be used for the benefit of mankind, it can also have serious detrimental consequences on the process of life.

BACKGROUND

In recent past, there has been continuing debate over the issue of interaction of radiation with matter. In the coming millennium, it is envisaged that there will be even more extensive industrial and medical exploitation of radiations. Therefore, the concern for human safety from damaging effects of radiation has progressively been increasing. With qualitative variations in radiations, notably high and low LET (linear energy transfer), different and some times contradictory results have been reported in the literature. Nonetheless, it is by and large accepted now that the interaction of low and high LET radiation with living system elicit different responses. Very low dose radiation have been shown to be beneficial and recent studies in the paradigm of low dose irradiation have lead to several new concept and creation of a sub-disciplines of study of radiation including hormesis (Sugahara *et al.*, 1998; Feinendegen *et al.*, 1999). The case of Kerala in our country is unique in terms of a large natural experimental laboratory. Kerala has very high background (natural) radiation exposing the population chronically to low doses of radiations. Various studies convincingly show that the effect is beneficial to human (Balram *et al.*, 1998). Radiation induced apoptosis or programmed cell death and induction of p53 have brought newer concepts to our understanding of radiation interaction with living system and newer paradigm have surfaced (Begum *et al.*, 1999). It appears that precise studies at molecular level are necessary to understand and reveal intricacies of effects of radiation. Better revelation shall lead to even enhanced application of radiation for the overall benefit of mankind and to improve the quality of life. Keeping this in view, it has been my endeavour for last over one and a half decades to understand the process of interaction of radiation with life so that beneficial aspects of radiation could be optimized. I have chosen for my studies different qualities of radiations (β radiation from (^{3}H) tritium in form of tritiated water, α radiation from ^{211}At (astatine), γ radiation from ^{60}Co and ^{137}Cs sources, neutron from baby accelerator and boron and lithium ions from Pelletron accelerator) to study how they interact with different components of the living systems at

molecular level both *in vivo* and *in vitro*. I intend to review briefly the experiences gained out of these studies without going deep into technical details.

Effects of low-dose tritium β exposure

Tritium (^3H) is a very weak β-emitting radioisotope of hydrogen. In form of tritiated water (HTO) ^3H can be a serious environmental pollutant in the future because it is produced in large quantities from industries based on nuclear technology. Since containment of such large quantities of HTO is practically not possible, it is released into the environment. Thus, it can become radiation source to which human and other form of life may get chronically exposed (Crowson 1973; Ei-Hinnawi, 1979; Sharan *et al.*, 1982). From radiation safety view point, the main concerns are (a) effectiveness of β particles of ^3H upon its decay (b) the relative biological effectiveness (RBE) which is accepted to be over 2, and (c) the ease with which HTO replaces normal body water (Sharan & Srivastava, 1980). I studied its effects using sensitive biochemical parameters after acute and chronic low-dose exposure. Acute exposure of swiss albino mice to ^3H as HTO at a dose of 0.0925 Mbq/ml lead to variable changes in the isozymes of hexokinase (a regulatory enzyme of energy releasing glucose metabolism) in brain and liver within 48 h of administration (Sharan & Srivastava, 1980). The results indicated possible conformational changes in the enzyme molecules upon β irradiation.

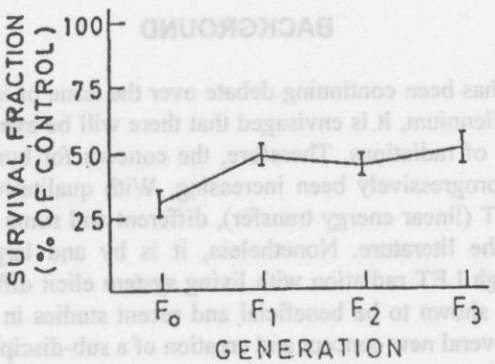

Fig. 1 : Plot of survival fraction in different generations of mice chronically exposed to HTO as drinking water (37 kBq/ml). The survival fraction was calculated by the CFU-S technique (From Srivastava *et al.*, 1982).

Table I summarizes the observations. The findings were similar after mice were exposed to chronic low dose HTO (37kbq/ml) for several generation even when the dose of irradiation was quite low; approximately between 41 mGy and 98 mGy (Sharan & Srivastava, 1984). However, on reconstitution of immune system in mice (assayed by spleen colony forming unit technique), what came to light was the fact that body has system to get acclimatized to low dose HTO exposure (Srivastava *et al.*, 1982). In other words, damages by HTO at the dose levels used in these studies could be taken care of by the system and no further damage was observed up to four generations that followed (Fig. 1). It must be kept in mind that exposure to HTO continued throughout. This fascinating capability of living system to get used to low dose radiation exposure (now known as Hormesis) appears to be an inherent quality of living system. Further, DNA strand break analysis was performed to see whether or not β rays from HTO affected DNA (by assaying single - and

Table I : Enzyme activity in different fractions in brain and liver of mice (From Sharan & Srivastava, 1980).

Tissue	Fraction	Isozyme Type	Activity of Enzyme[1] (μmoles of NADPH formed/min at 37°C) Time after tritiated water treatment (in hours)					
			Control	2	4	12	24	48
Brain	Pellet	I	2.4367 ±0.2060	2.1550 ±0.0518	2.0268 ±0.0323	2.9757 ±0.0665	1.7910[a] ±0.1311	3.9923[c] ±0.0483
		II	0.0916 ±0.0375	0.2100 ±0.0612	0.1229 ±0.0724	0.2908[b] ±0.0474	0.4566[c] ±0.0185	0.1236 ±0.0169
	Supernatant	I	0.8664 ±0.0981	0.6000 ±0.0108	1.1916 ±0.0035	0.8968 ±0.0167	0.7020 ±0.0154	1.2411[a] ±0.0162
		II	0.3255 ±0.0432	0.3750 ±0.0119	0.2772 ±0.0467	0.1327[c] ±0.0051	0.1467[c] ±0.0192	0.2994 ±0.0379
Liver	Supernatant	I	0.2204 ±0.0680	0.0233[a] ±0.0032	0.3168 ±0.0000	0.1246 ±0.0349	0.1476 ±0.0115	0.4578[b] ±0.0107
		II	0.5730 ±0.0359	0.4237[b] ±0.0310	0.4212 ±0.0490	0.6044 ±0.0434	0.6969 ±0.1094	0.8965[c] ±0.0122
		IV	0.3769 ±0.0741	0.5700 ±0.0334	0.8064[b] ±0.0000	0.3308 ±0.0198	0.0804 ±0.0023	0.7351[b] ±0.0829

1= Mean ± S.E. ; a=p>0.05; b=p>0.02; c=p>0.01.

double stranded DNA breaks) in mice exposed to it (Srivastava *et al.*, 1982). Double stranded DNA breaks kept increasing significantly 1st generation onwards. Biological effects were also observed on ovarian cells in mice exposed acutely or chronically to low dose HTO (Kapoor *et al.*, 1985). These results indicated that β rays inflicted dose dependent radiolesions.

An important regulatory class of chromosomal protein is the high mobility group (HMG) protein which are involved in organization of chromatin and regulation of gene expression (Nicolas & Goodwin, 1982). Chronic low dose exposure of mice tc HTO was found to be toxic to liver HMG proteins (Sharan *et al.*, 1996a). The analysis of results revealed that the acetone precipitable HMG proteins from mice liver was significantly inhibited in F_1 mice chronically exposed to HTO (Fig. 2). The observations reflect radiotoxicity of β particle at molecular level in

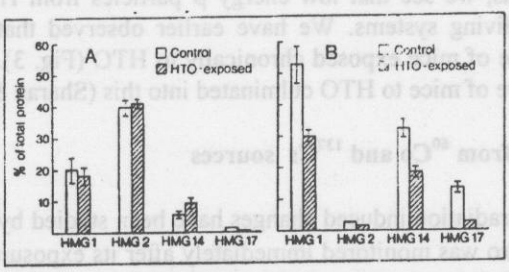

Fig. 2 : Densitometric quantification of liver HMG proteins of F_1 mice chronically exposed to HTO as drinking water (37 kBq/ml). Panel A shows acetone HCl fractions while panel B shows the acetone fraction of HMG proteins (From Sharan *et al.*, 1996a).

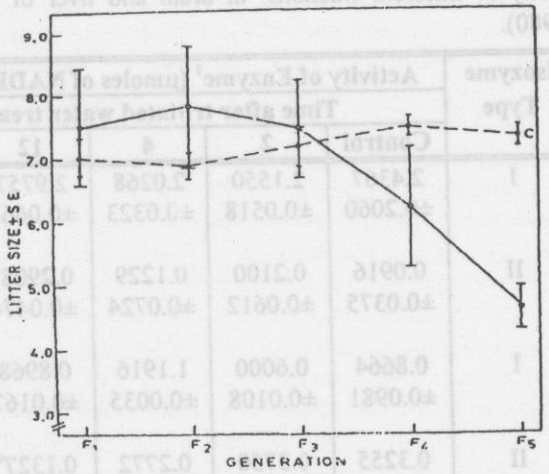

Fig. 3 : Variation in the size in different generations of untreated mice (broken line) and after chronic exposure to HTO as drinking water (37 kBq/ml) (From Sharan & Srivastava, 1984).

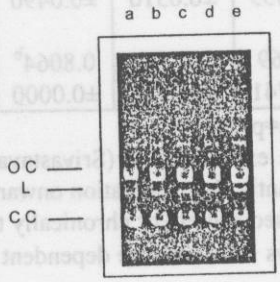

Fig. 4 : Agarose gel electropherogram of unirradiated plasmid DNA (lane a), γ irradiated plasmid DNA samples (lanes b, c, d & e for 30, 60, 120 & 240 Gy respectively); CC=closed circular, OC=open circular, L=linearized forms of plasmid (From Humtose *et al.*, 1998a).

a biological system. Thus, we see that low energy β particles from HTO at a low dose affects various components of living systems. We have earlier observed that there was a progressive reduction in the litter size of mice exposed chronically to HTO (Fig. 3). It appears that molecular events following exposure of mice to HTO culminated into this (Sharan & Srivastava, 1984).

Effects of γ irradiation from ^{60}Co and ^{137}Cs sources

Several aspects of γ radiation induced changes have been studied by my group. The radiolysis of enzyme catalase in vitro was monitored immediately after its exposure to up to 80 Gy of γ-rays from ^{60}Co. The enzyme activity generally declined with increasing radiation dose (Wary & Sharan, 1988). In human lymphocytes, the γ irradiation caused a dose dependent increase in DNA strand breaks and correspondingly reduced survival of the cells *in vitro* (Wary *et al.*, 1989; Sharan,

1990). At molecular level the effects have been monitored at several parameters. The liver acetylcholine esterase enzyme activity was enhanced several folds by whole body irradiation of mice (Sharan *et al.*, 1995). The status of poly-ADP-ribosylation reaction in mice was also followed (Sharan *et al.*, 1996b). Poly-ADP-ribosylation is an important post-translational modification of chromosomal proteins (Althaus & Richter, 1987). The reaction strongly influences chromatin organization and may have potentials of regulating DNA repair and gene expression (De Murcia & De Murcia, 1994; Boehm *et al.*, 1997). This parameter has been used to monitor radiation induced damages and their repair. Using human kidney TI cell line, it has been shown that γ radiation up to 3 Gy from ^{137}Cs source caused and maintained significant decrease in total cellular poly-ADP-ribosylation (Sharan *et al.*, 1996b) while poly-ADP-ribosylation of individual histone proteins H3, H1and H2B were enhanced (Sharan *et al.*, 1998). It has been shown that the intrinsic affinity of histone to DNA was altered, perhaps due to poly-ADP-ribosylation of histones. Similarly, ability of γ rays from ^{60}Co source to cause damage to DNA was directly demonstrated in a bacterial plasmid system (Humtsoe *et al.*, 1998a). Dose dependent significant increase in the relaxed form of plasmid, signifying occurrence of single strand breaks, and practically no double strand break was demonstrated for doses of irradiation up to 240 Gy (Fig. 4). Using restriction endonucleases it has been shown that other qualities of damage induced in the plasmid was dependent on the nucleotide sequence wherein GC-rich nucleotide sequences were more vulnerable to radiation induced changes (Humtsoe *et al.*, 1998a).

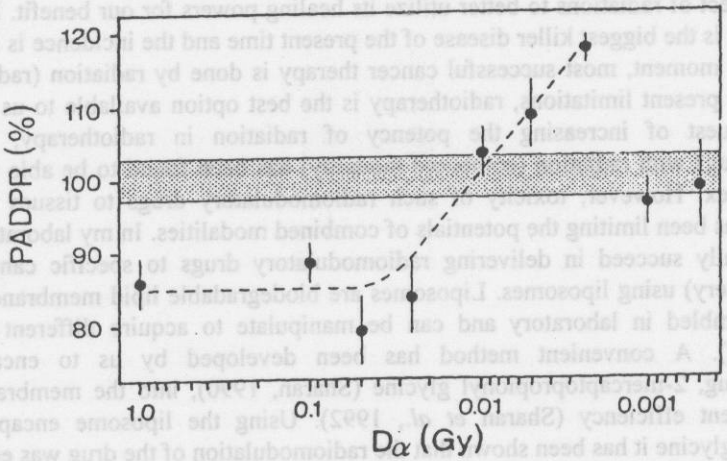

Fig. 5 : Poly-ADP-ribosylation of total cellular proteins in human glioblastoma cells as a function of absorbed α dose from extracellular ^{211}At. The normal level of poly-ADP-ribosylation is shown in the shaded area which has been taken as 100% (From Schneeweiss *et al.*, 1999).

Effects of high LET radiations on living system

In an effort to go deep into the molecular mechanism of radiation induced changes, the effect of heavy ions has been studied using different cell systems. Contrary to effect of low LET radiations like γ rays, neutrons (Baby Cyclotron, Juelich, Germany) caused initial depression followed by dose dependent recovery of total cellular poly-ADP-ribosylation (Sharan *et al.*, 1996b) and the enhanced level of poly-ADP-ribosylation was interpreted as favorable for repair

processes. Similar studies using α rays from ^{211}At on human Glioblastoma cell line 86 HG-39 showed that for α dose range between 0.001 and 0.015 Gy the level of poly-ADP-ribosylation was either normal or exaggrated (Fig. 5) suggesting the possibility of repair of high LET radiation induced damage in this dose range (Schneeweiss *et al.*, 1999). Dose higher than this lead to expected lowering of poly-ADP-ribosylation and, therefore, not favoring of induced damages.

Using different human and mouse cell lines it has been shown that boron ion irradiation from a Pelletron accelerator affected human and mouse cell lines differently in terms of survival of cells, acetylcholine esterase activity, and membrane damages (Sharan *et al.*, 1996c). The results also suggested that the response of mouse and human cell lines to heavy ions were different. Another heavy ion, lithium (^{7}Li), affected bacterial plasmid and caused dose dependent damage to the DNA which was qualitatively different from the damage recorded by low LET radiation (Humtsoe *et al.*, 1998b). These results corroborate our earlier contention that damage to plasmid DNA could be influenced by the nucleotide sequence. The possible implications of these observations are significant and may have answer to the molecular mechanism of genome instability and genetic pre-disposition, phenomena hitherto not understood. More work is under progress on this line.

Radiation in cancer radiotherapy

A proper understanding of interactions of radiations with molecules of living system can potentially improve medical application of radiations. In recent years we have been engaged in elucidating this aspect of radiations to better utilize its healing powers for our benefit. It is known that globally cancer is the biggest killer disease of the present time and the incidence is on a rise in our country. At the moment, most successful cancer therapy is done by radiation (radiotherapy). Notwithstanding its present limitations, radiotherapy is the best option available to us for cancer therapy. In the quest of increasing the potency of radiation in radiotherapy, combining radiomodulatory drugs with radiation (combined modality) has been found to be able to increase the therapeutic index. However, toxicity of such radiomodulatory drugs to tissues other than cancerous tissues has been limiting the potentials of combined modalities. In my laboratory, I have been able to partially succeed in delivering radiomodulatory drugs to specific cancer tissues (targeted drug delivery) using liposomes. Liposomes are biodegradable lipid membrane bags that can be easily assembled in laboratory and can be manipulate to acquire different properties (Gregoriadis, 1988). A convenient method has been developed by us to encapsulate a radiomodulatory drug, 2-mercaptopropionyl glycine (Sharan, 1990), into the membranous bags with high entrapment efficiency (Sharan *et al.*, 1992). Using the liposome encapsulated 2-mercaptopropionyl glycine it has been shown that the radiomodulation of the drug was enhanced in normal mice (Sharan *et al.*, 1995) as well as in cancer bearing mice (Chakraborty, 1997; Alam *et al.*, 1999). While more work continues on the optimization of the targeted drug delivery, it has been demonstrated convincingly that such an approach shall increase clinical efficacy of radiotherapy in the future.

FUTURE PERSPECTIVES

While a lot has been learnt about interaction of radiations, particulary of low LET quality, with living systems it appears that there are fascinating molecular aspects of such interaction that remain gray areas. Relatively little is known about the interaction of high LET radiations with living systems. Since, radiations are capable of interacting with biomolecules it is important to understand the molecular mechanisms of interactions for optimal utilization of radiation for the

improvement in quality of human life. Radiation induced apoptosis, low dose radiation effects and hormesis, genetic pre-dispositions are some of several fascinating aspects that await elucidation. Role of repair genes and radiation induced immune responses are other aspects that can seriously influence the way we look at radiation. A proper understanding of the complex interactions of radiation with matter can potentially pave way for its use for the betterment of quality of human life in the coming millennium. The destructive powers of radiations can not be ignored. Only a proper understanding of this can make radiations increasingly more useful to mankind.

ACKNOWLEDEMENTS

I am thankful to my colleagues in India and abroad and to my students who have contributed to this review. Thanks are due to funding agencies UGC, CSIR, DST and Indo-German R & D Cooperation Program who have provided funds for some of the studies mentioned in this review. Heavy ion irradiation facilities were kindly provided by NSC, New Delhi and FZ, Juelich.

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