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Cytogenetic aspects of neutron-induced cellular response

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Abstract: The RBE values for neutrons vary to a wide range and the influencing factors have been discussed. The relevance of cytogenetic end points as signature of the past neutron exposure, and the importance of cosmic component of neutron have been explored. The neutron-induced multiple damage sites are understood as more mutagenic, carcinogenic and lead to complex chromosome aberrations. Low energy neutrons (0.2- 2 MeV) are the principal concern in biodosimetry. They exhibit little or no ß component and also independent dose-rate effect. The review of also emphasizes the wide range of RBE need to be considered for neutrons. Such knowledge may be helpful to draw guidelines to set dose limit for radiation workers under specific field and to devise effective neutron therapy. Also to make high-altitude travels and space voyages safer for human beings.

Key words: Neutrons, RBE, adaptive response, dsb, radiation, chromosomal aberrations

1. Introduction

the relative biological Study of effectiveness (RBE) of neutrons is important for risk assessment and in understanding the basic mechanisms of radiation biology. Also, the health effect of neutron exposure has been a great concern ever since the 'Little Boy' was detonated over Hiroshima in 1945. It gains momentum in tune with further space research, high altitude commercial operation and incident like 'Tokai Mura' (Blakely, 2002). In the past, the epidemiological data for human exposure to neutron has relied heavily on the data of Abomb survivors. The older "T65D" (tentative 1965 dose) estimates predicted a substantial neutron component to the doses received by the survivors in Hiroshima. Accordingly, a high estimate of neutron RBE had been calculated for leukemia (RBE_M≈60). But when the doses are reassessed in 1986 (DS 86), the contribution of neutron to the absorbed total doses was downgraded to 1/10 of the T65D dose estimate (Ruhm et al., 1998). As a consequence, there is little information on neutron RBE in relation to cancer or any other end points from the direct human epidemiological data (Little, 1997). At this juncture, neutron effect has to be understood largely from the experimental studies involving laboratory animals, plants, blood cells and cell lines (ICRU, 1986; ICRP, 1991; NCRP, 1990). The response is studied as different end points (markers) and measured as relative biological effectiveness (RBE) in comparison with a

reference radiation (usually, 60 Co– γ / 137 Cs- γ or X-ray (250 kVp). The neutron RBE for variety of experimental end points, at low dose limit, ranged from 2-100 was reported by NCRP (1990). In this review, an attempt has been made to highlight how the neutron-induced genetic damage is understood as wide range of RBEs, even though the number of initial strand breaks is believed to be unison for different radiation at a given dose (Nikjoo et al., 1998).

2. Cytogenetic damage and biodosimetry

The study of chromosome aberration (CA) frequencies in stimulated peripheral blood lymphocytes serves as biological dosimeter to probe accidental radiation exposure. The large neutron data, pertaining to human response, is derived from such in vitro studies where blood T-lymphocytes can readily be induced to grow outside the body by adding phytohaemagglutinin (a plant lectin) and the cell growth can easily be arrested at appropriate stage of the cell division to make chromosome analysis feasible under light microscope. Generally, spindle-poisoning agent such as colcemid is used to arrest cells at metaphase. Cytochalasin-B is used to arrest at cytokinesis for micronuclei analysis. The whole method is non-invasive as the analysis is done out side the human body. It also meets the bioethic norms as it is generally carried out after informed consent of the donors. The in vitro dose-response curves generated out of it have led to the assessment of RBE of different radiation (NCRP, 1990) which contributed to set

Table 1. Wieghing factors for different neutron energies and other radiation

Type & Energy range	Radiation weighing factor (W _R)
Neutron energy: <10 keV	5
>10 keV to 100 keV	10
>100 keV to 2 MeV	20
>2MeV to 20 MeV	10
>20 MeV	5
∝-Particles, fission fragments, heavy nucleus	20
Proton, other than recoil proton, energy >2 MeV	5

the quality factor (Q) for neutrons (Table 1) of different energies. The radioresponse of blood sample is believed to be the same both at in vivo and in vitro irradiation. The radiation induced chromosome breakage rates are virtually the same for different mammalian species and for different individuals. The



dicentric assay (Fig.1) can be used to estimate

Fig. 1 Mitotic spread of irradiated human lymphocyte showing dicentric chromosome (IAEA, 1986)



doses as low as 0.02 Gy when large number of metaphases is scored. Generally, it takes 1 day to score few hundred metaphase cells. The labor can be reduced on automation using computer software. Dicentrics are unstable aberrations and can disappear from the blood with passage of time. The mean half time of dicentrics is 3 years (IAEA, 1986) but much less value of 110 day has been calculated after analyzing the Goiania accident victims (Ramalho *et al.*, 1995). Dicentric analysis is the method of choice after an acute over-exposure of small number of individuals. The micronucleus assay (Fig. 2) coupled with FISH

Fig. 2 Irradiated cell exhibits micronucleus



(fluorescent in situ hybridization) technique is comparatively easier for analysis. The detection limits of 0.1 to 0.2 Gy can be achieved by scoring about 2000 BN cell, which takes 1/2day. But a decline in the MN frequency of about 60% after 1 year has been observed in radiotherapy patient (Fenech et al., 1990). Certainly, MN assay allows rapid screening of accident victims. Analysis of chromosomal translocations by FISH is suitable to study over-exposure cases and chronic for retrospective dosimetry. Since translocation is correlated to neoplastic transformation, this technique gains importance in the field of radioprotection.

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3. RBE of neutrons and the influencing factors

The neutron RBE values for chromosomal damage (dicentric and centric rings) in human peripheral lymphocytes are usually much higher (Scott et al., 1969; Loyd et al., 1976) than those for mutation measured at hypoxanthine-guanine phosphoribosyl the transferase locus in the mammalian cells (Richold & Holt, 1974). Neutrons were shown to be more efficient than both X-ray and gamma rays in inducing oncogenic transformation in rodent cell (Borek et al., 1978; Hall et al., 1982; Han & Elkin, 1979). The variability in induction of chromosome aberrations (CA) among five mammalian species was reported. Taking the frequency of dicentrics in lymphocytes of man 1, the relative frequency in each species was examined as 0.79, 0.24, 0.22 and 0.16 in monkey, rabbit, cat and dog respectively. The interspecies differences were attributed to the differential response of T-lymphocytes in each species probably related to evolutionary scale (Muramatsu & Matsuoka, 1976). At the same time, chemical environment (Pogozelski et al., 1999) and the cellular tissue component (Meijne et al., 1992) can also influence the radiation-induced damage. The RBE of neutrons becomes reduced when CA are studied on human sperm chromosome using in vitro fertilization system. This discrepancy was attributed to the DNA-repairing capacity of oocytes (Tateno et al., 1996).

A (dose)² component reported for acute doses of low LET radiation either absent in neutron radiation or present little. The CA induced by 25 keV neutrons in human lymphocytes gave linear dose response curve of 1.1 dicentrics/cell/Gy (Aghamohammadi *et al.*, 1989). In Chinese hamster splenocytes, 1 MeV fast neutrons increased dicentrics in a linear fashion with RBE of 5-8. While the response was linear-quadratic for X-rays (Grigorova *et al.*, 1998). Pandita and Geard (1996) recorded the dose response for dicentrics was always linear for the energy range between 0.22 and 13.6 MeV of monoenergetic neutrons (RARAF, Columbia).

The "Little Boy"-replica (LBR) assembled at Los Alamos as a controlled nuclear reactor has served for studying the biological effectiveness of actual Hiroshima neutrons in human blood lymphocytes exposed in vitro (Dobson et al., 1991). This study provided the first direct measurement of the biological effectiveness of neutrons having very similar energy characteristics to those received by survivors of Hiroshima bomb of 1945. The dose response curve for the induction of dicentrics and ring by LBR neutrons was linear and in contrast to the linear-quadratic response of γ -ray. The RBE_M values for LBR neutrons are





RBE varies with neutron sources. A value of 8 was obtained when considering the ratio of α - coefficient for high-energy neutrons (600 MeV) and γ -rays, whereas a value of about 50 is obtained for fission neutron (0.4 MeV) (Vulpis, 1982). Sreedevi *et al.*, (1997) studied the induction of CA and MN in human peripheral blood lymphocytes irradiated with fission neutron from a research reactor (50 kW) and compared it to that of 250 kV X-rays. The RBE for MN and CA were 10.3 and 25.3 respectively (at doses <0.5 Gy).

Experiment with monoenergetic neutrons:

People exposed to neutron of wide energy ranging from hundreds of keV to few MeV and it is imperative to consider neutron RBE over the energy range. At the same time, RBE values among different neutron sources strongly indicate its energy dependency. Hence, the question of biological effectiveness can be addressed most readily by examining the consequences of exposures to monoenergetic neutrons. Miller et al. (1989) studied RBE of a range of neutron energies relative to X-rays for oncogenic translocation and cell survival in the mouse C3H 10T1/2 cellline. Monoenergetic neutrons ranging from 0.23 to 13.7 MeV was used at low doses 0.05 to 1.47 Gy. RBE of neutrons over different http://www.indjst.org V

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energies was found linear and inversely related to dose: while X-ray exhibited curvilinear response. Maximal RBE values for transmutation ranged from 13 for cells exposed to 5.9 MeV neutrons to 35 for 0.35 MeV neutrons. The RBEs for both transformation and survival were comparable. Earlier, Hei et al. (1988) used the same neutron facility (RARAF, Columbia Univ.) for neutron energy ranging from 0.33 to 14 MeV and compared with 137 Cs- γ Cytotoxicity and mutation Cytotoxicity and mutation frequency in the AL cells (human-hamster hybrid) was studied. The induction of mutagenesis by neutron was found to be energy dependent but the frequency was curvilinear. RBE for cell lethality at 10% survival ranged from 5.2 for 0.33 MeV to 1.8 for 14 MeV neutrons. The RBE for mutation at a1 locus ranged from 30 for 0.33 MeV to 4.2 for 14 MeV. In both experimental studies, the pattern of response was same, but the RBE value changed according to the end point and system studied. The variability of neutron RBE was also observed by Pandita and Geard (1996) using the same neutron facility. When normal human fibroblasts were irradiated, a linear dose response (Y= α D) for CA was obtained for all monoenergetic neutrons and for 137 Cs- γ -rays. The yield of CA per unit dose was high at low neutron energies (0.22, 0.34 and 0.43) with a gradual decline were noticed with increasing energy of neutrons. Maximum RBE (RBE_M) values varied for the different types of CA. The highest RBE (24.3) for 0.22 and 0.43 MeV neutrons was observed for intrachromosomal deletions. Even for the 13.6 MeV neutrons the RBE_M exceeded 10. These results also reveal that RBE of neutron varies with energy and dissimilar between different types of asymmetric CA. The proportion of dicentrics, centric rings, interstitial deletions and terminal γ-ray-induced deletions relative to all aberrations was 45.6, 4.1, 39.6 and 10.6% respectively. For the same aberration types, the range for the various monoenergetic neutrons observed was 30.6-34.2, 1.6-6.6, 43.2-52.7 and 9.3-15.5% respectively. Geard (1996) irradiated Vicia faba cells with monoenergetic neutrons of 230, 320, 430 and 1910 keV and recorded CA. Neutrons of 320 keV were the most efficient at aberration production per unit dose, followed by 230 and 430 keV neutrons (equally effective), and then 1910 keV neutrons. A linear response was recorded among energy ranges, some are more effective at some type of aberration induction per unit absorbed dose. For example, 230 keV neutrons in the above study are most effective in inducing chromatid interchanges, owing to the productions of its greatest relative frequencies of short (<1 µm) proton tracks with greatest effective which produce

"Neutron-induced cytogenetic damage"



aberrations most likely result from interaction of intimate loop association in a chromatid. Hence considering RBE of particular end point for particular radiation may not reflect the actual biological impact potential of the radiation.

The rate of induction of mutation is not only neutron energy dependent but also relies on the type of mutation locus studied. In Chinese hamster V79 cell line, the frequency of induced mutants at the *tk* gene was higher than

at the hprt gene. The RBE value for 14 MeV neutrons was 5.4 for the hprt locus and 36.6 for TK normal growth mutant (TK_{ng}) when 137 Cs- γ was used as standard (Zhu and Hill, 1994). The type of reference radiation influences the outcome of RBE value. X-ray and 60 Co- γ rays differ in effectiveness by a factor of 2-3. Accordingly, the RBE value tends to be greater by a factor of 2-3 for more energetic photons are used for reference. Thus, consistent differences RBE in were demonstrated.

Neutron irradiation leads to life shortening and induces more neoplastic transformations. А longevity involving Rhesus monkeys after X-rays and neutron irradiation relativelv suaaests the hiah effectiveness of fission neutrons (RBE >10). Similar experimental results with X-rays and fission neutron on hybrid mice yielded RBE of 2-7 for life shortening (Ainsworth et al., 1976). Thus, with respect to cytogenetic damage, survival, mutagenesis and neoplastic transformations, it is well established that neutrons are more effective than low LET radiation such as X- and γrays. At the same time, RBE of neutrons varies (Table 2) very much depending upon various factors such differences as in endpoint (UNSCEAR, 1988), study system, source of neutrons, type of reference radiation and dose.

Molecular mechanism of high RBE

Is there any quantitative or qualitative difference in neutroninduced DNA damage which is the initial step for all these aberrations?. Information, particular to neutrons, is scanty, although sufficient information is available on high LET radiation. Many studies have shown that in spite of increase in LET, radiation generally shows the RBE close to unity for dsb induction despite the increase in RBE for cell killing. RBEs of about 1 for DNA-dsb induction in V79 cells in a dose range of 1-20 Gy of fast neutron have been observed (Nikjoo *et al.*, 1998; Prise *et al.*, 1987; Kysela *et al.*, 1993a). A linear relationship between dsb and lethal lesions was found. But neutron induced dsb were 2.5 times more effective than that of 250 kV X-rays (Prise *et al.*, 1987). However, recent studies show that high-LET radiation generates

Table 2. Neutron-RBE for different end points

End point	RBE	Reference
Chromosomally	1.6	Tateno <i>et al.</i> , 1996
abnormal		
spermatozoa		
Chromosome type	1.6	Tateno <i>et al.</i> , 1996
breaks		
Spermatozoan with	2.0	Tateno <i>et al.</i> , 1996
CA		
Chromosome type	3.2	Tateno et al., 1996
exchange		_
Chromotid type	3.9	Tateno <i>et al.</i> , 1996
breaks		
PCC fragment	~2-2.4	Loucas & Geard, 1994;
		Prasanna <i>et al.</i> , 1997
Leukaemia	~2	Prasanna <i>et al</i> ., 1997;
		Major & Mole, 1978;
		Mole & Davids, 1982;
		Upton <i>et al.</i> , 1970.
Translocations	4.22	Muramatsu <i>et a</i> l., 1973
Dicentrics	2-5	Gooch <i>et a</i> l., 1964
Mutation (A _L mutant)	4.2-30	Hei <i>et al</i> ., 1988
Cell survival	5.2	Hei <i>et a</i> l., 1988
Mutation (HPRT)	5.4	Zhu & Hill, 1994
Mutation (TKng)	36.6	Zhu & Hill, 1994
Ovarian tumour	~8	Fry, 1981; Ullrich, 1983
Tumour induction	>10	Van Zwieten <i>et al</i> , 1978
Transformation	13-35	Miller <i>et al</i> ., 1989
Lymphoma	14	Fry, 1981; Ullrich <i>et al</i> .,
		1976
Dicentrics & rings	10.9	Tanaka <i>et al.</i> , 1994
Reciprocal		
Translocation,	5-84	Grahn <i>et al</i> ., 1984
Preimplantation loss,		
Testis Weight loss,		
Abnormal sperm		
morphology		
CA	24.3	Pandita & Geard, 1996
Reduction in seedling	6.7-36	Gopal-Iyengar <i>et al.</i> ,
height		1974
Eye lens opacification	6-24	Di Paola <i>et al</i> ., 1980
in mice		E 1001 1
Mammary carcinoma	33	⊢ry, 1981; Ullrich, 1983
Lite shortening	40	I nompson <i>et al.</i> , 1981
Pituitary tumour	59	⊢ry, 1981; Ullrich <i>et al.</i> , 1976
Lung carcinoma	60	Frv. 1981: Ullrich <i>et al.</i>
		1976
Dicentrics	22-80	Dobson <i>et al</i> ., 1991
Lung adenomas	200	Fry, 1981; Ullrich <i>et al</i>
		1976

"Neutron-induced cytogenetic damage"

DNA fragments of smaller size than X-ray or γ ray. Newman et al. (1997) observed the DNA breakage pattern in Chinese hamster V79 cells after irradiation of X-ray or α -particles from ²³⁸Pu source using pulsed-field col electrophoresis (PFGE). α-particle generated higher numbers of fragments below 0.3 Mbp in size and lower number of fragments above 0.7 Mbp, non-randomly, compared to X-ray. Thus, high LET radiation including neutrons differ by producing closely packed fragments in spite of the total number of fragments remain unity for both high and low-LET radiation. Blazek et al. (1993) using a modified filter elution technique, step elution, reported that dsb produced in cells by fast neutrons are clustered when compared to those produced by gamma. Recent developments in PFGE allow the measurement of both the vields and the distribution of breaks within the genome, which go part of the way to explaining the RBE values close to 1 previously measured using other approaches with various radiation quality (Prise et al., 1998). A study conducted on the non-cycling human fibroblasts, immediately after irradiation to monoenergetic α -particle, results in the RBE of 2 when excess PCC fragments scored against X-rays (Loucas & Geard, 1994). When CA was scored at metaphase, the RBE considerably increased. It is inferred that greater aberrations recorded after high-LET radiation are not principally attributed to an increase in breakage efficiency. The interaction between breaks along the same particle track was considered to be important. Thus the process involved in the conversion of DNA lesions to cellular effects must be dependent on LET. RBE values for dsb induction do not reach high values, but are generally less than 3, contrasting with the much larger values obtained for reproductive death of cells, CA and mutations (Brenner & Ward, 1992). RBE of neutrons in mice exposed in vivo (14 MeV Neutrons at single doses of 2 cGy to 3 Gy in comparison with $^{60}\text{Co}~\gamma$ rays) for 50% reductions of weight, testis primary spermatocytes, and elongated spermatids was found to be 2.5, 10.0 and 6.1, respectively (Hacker-Klom et al., 2000).

Neutrons preferentially produce breaks that are very close together. When ionizing radiation strikes a cell, dsb and other lesions are produced within less than millisecond. The signal generated by the dsb would trigger battery of enzymes to cut and recombine DNA either within or between chromatids. If this process of repair interrupted by entry of the cell into mitosis with concomitant chromatin condensation, an incomplete exchange would result, leading to a visible chromatid break. Simple lesions do not play role in the biological effects of ionizing radiation since DNA repair http://www.indjst.org Vol.1 No.1 (Nov. 2007)

systems are highly efficient for the repair of such DNA modifications. In contrast, the main biological relevance of radiation induced base damage would be due to their role in multiple lesions such as LMDS (locally multiply damaged sites). Neutron produces more severe lesions than γ -rays by mainly one-track mechanism. The majority of DNA damage lesions caused by low LET are of simple type, while the total yield of strand breaks remain constant; and at high LET values nearly 70% of all dsb are of complex type (Nikjoo et al., 1998). High LET radiation produces increased complexity of lesions due to the formation of LMDS (Ward et al., 1995). These are formed by the highly localized depositions of energy, which are produced as densely ionizing tracks interact with the DNA. They consists of multiples of base damages and breaks produced on the strands of the DNA within the distance of <20 kb. These lesions will be indistinguishable from simple dsb in the current assays available; however, differences in the rejoining of dsb with increasing dsb complexity (Prise et al., 1994).

Generally the number of dsb does not indicate the degree of complexity of DNA damage. But α dsbMDS component is suggested to mark the complexity. A direct support comes from an elegant work on the influence of OH scavengers to modify the DNA insult borne by pBR322 plasmid DNA in aqueous condition. The increasing OH scavenging capacity with the decrease in yields of strand breaks for fission neutrons was not as pronounced as in 60 Co- γ rays. It is reasoned that neutron produces tracks of closely spaced radicals combine to cause more complex damages when compared to the sparsely ionizing radiation (Pogozelski et al., 1999). Double strand breaks produced by high LET radiation are more "severe and long-lived" (Sachs & Brenner, 1993). What we call dsb can most likely be a heterogenous mixture involving a spectrum of lesions arising from ionizing events and subsequent chemical reactions on DNA in the nuclear milieu. It could be simple dsb resulting from 2 single ionization in close proximity on opposite DNA strands or cluster of localized multiple damaged sites as a consequence of energy deposition by high LET radiation (Kysela et al., 1993b). This can be explained with Monte Carlo track structure simulations resulting in different ionization pattern for different types of radiation with more energy deposited in large clusters for high LET radiation (Goodhead, 1989), also increased lethality of dsb observed with increasing LET of radiation (Prise et al., 1990) and that generally less (slowly) rejoining can be found with high LET radiation (Kysela et al., 1993a). This may

result in increased frequencies of incomplete exchanges induced by the neutrons. Dsb induced by high LET radiation are less easily rejoined than those induced by low LET radiation with more residual and unrepaired breaks rejoining. This has been shown in premature chromosome condensation (PCC) studies following neutron irradiation of human lymphocytes (Darroudi et al., 1997). Dsh induced by neutrons rejoin more slowly. Thus, increasing the slow repair component and giving rise to the formation of higher frequency of insertions and more complex aberrations, which forms the base for the neutron fingerprint. In a biologically active system, repair enzymes will considerably modify the original damage (chromosome aberrations are mainly by misrepair), whereas in a passive system, such as purified DNA, the damage may be closely associated with initial pattern of energy deposition. In the same way, the response of cell line, which lost cell-cycle control, must be different from the response of blood lymphocytes. The quality of initial DNA damage and the extent to which they are repaired or misrepaired matter for the difference in RBE found between high and low-LET radiation (Vral et al., 1998).

Particles of same LET but different in charge and velocity produce altered pattern of energy deposition in the target material. Thus physical differences are relevant to the type of biological damage produced. Recent experiment shows that charged particles with the same LET produce different yields of CA. Also their yield would very well reflect differences in pattern of energy deposition in the nucleus and /or of the different chemical nature of the DNA lesions (Durante et al., 1998). It is clear that future studies to determine the effectiveness of radiation of differing LET must use technique that determine yield and distributions of dsb, and assays need to be developed to allow these measurements at biologically relevant doses. Any experiments that integrate initial induction and rejoining endpoints will be useful. Alkaline comet assay has recently been introduced as most sensitive and rapid technique to probe DNA damage at individual cell levels in biomonitoring as well as in cancer treatment (Olive, 1999). Those cells carrying high radiation insult unable to grow under conventional techniques can also be used. This technique accommodates the heterogeneous population of radiation sensitive and resistant cells to study both single strand and double strand DNA breaks. The "tail moment" is a popular method of comet evaluation, which incorporates both the amount of damaged DNA in the tail and the distance it migrates

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depending upon the fragmented size. Though comet assay cannot reveal the population- and size- of dsb, an arbitrary assessment always possible based on the mobility difference in an agarose bed. Tiny DNA bits migrate faster and take the tail end. Thus the free end of the tail in alkaline comet assay is considered to have the DNA population after dsb. To gain an insight into the induction of initial DNA damage and the relevance in their subsequent formation into chromosome aberrations, Gajendiran et al. (2000) irradiated human peripheral blood lymphocytes with a range of monoenergetic neutrons and studied both the aspects simultaneously. Monoenergetic neutrons at 2.3, 1, 0.79, 0.57, 0.37 and 0.1867 MeV emitted from HIRRAC (Hiroshima University Radiobiological Research Accelerator) which

Fig. 4 Monoenergetic neutron-induced initial DNA damage as measured by comet assay (Gajendiran et al., 2000; Tanaka et al., 1999)



approximate the energies produced by the atomic bomb used at Hiroshima, were used. ^{252}Cf as fission neutrons were served for comparison while $^{60}Co-\gamma$ was used as reference to obtain RBE. Irradiated blood lymphocytes

Fig. 5 RBE of monoenergetic neutrons (Gajendiran et al., 2000; Tanaka et al., 1999)



were shared for comet assay (to study initial DNA damage) and for chromosome aberration studies (Tanaka *et al.*, 1999) (late effect of DNA strand break). The results reveal that the finger print left in cells exposed to neutrons differing only by narrow energy range can be detected by comet assay (Fig. 4). Neutron of different energy range varied in their initial-DNA-damage



pattern, which was reflected in the form of differences in tail moment. Low energy neutrons generated lengthy comet tail composed mostly of tiny DNA bits obviously due to clustered damage. PFGE data also confirms clustered dsb after high LET persons. irradiation (Sachs et al., 1998). This was quite contrast to the short tail of uniform streaking, generated by γ -radiation. Comet assay was performed under conditions (alkaline pH 13; 1 h lvsis: 20 min rewinding: electrophoresis at 300 mAmp for 20 min under dim light) suitable for making comparative study among wide range of doses with different LET. Under these conditions, the sensitivity of comet assay to detect absorbed doses covered 0.125 to 0.5 Gy of neutrons, which exhibited liner response. The RBEs of monoenergetic neutrons based on comet assay were 6.3, 5.4, 4.7, 4.3, 2.6, 1.7 for 0.37, 0.57, 0.79, 0.186, 1 and 2.3 MeV neutrons when 60 Co- γ was used as reference. the normal cells. Peak biological effectiveness was found at 0.37 and 0.57 MeV. The effect came down with further decrease or increase from the peak energy level (Fig. 5). The RBE observed by

comet assay is relatively lower than RBE observed for CA. Chromosome aberrations produced by the same monoenergetic neutrons are shown in Fig. 6. The response pattern was similar to that of comet assay but the RBE values were high for CA. A linear dose response was observed for CA at all energy levels. For dicentrics, RBE values were 11.2, 16.4, 10.7, 7.1, 7, 3.9 for 0.186, 0.37, 0.57. 0.79, 1, 2.3 MeV coinciding with energy dip. The RBE value showed an upward trend from ²⁵²Cf neutron to a peak value at 0.37 MeV, but the value decreased to 11.2 at 0.186 MeV. The response pattern of initial DNA damage and chromosome exchange was quite similar.

However, acentric rings showed no significant differences among monoenergetic neutrons, even though the highest RBE was obtained at 0.37 MeV neutrons. Since comet assay senses DNA damage only, the higher RBE value in other end points suggest that the biological effectiveness becomes amplified after initial damage due to cell proliferation after irradiation. The degree of amplification seems to be neutron energy dependent. This assay can be helpful to screen samples rapidly, especially at the time of accidental exposure for speedy medical care. It should also be noted that in Hiroshima 65% of the neutrons have been estimated

to be between 0.1 and 1 MeV. Straume et al. (1991) estimated that Hiroshima neutrons were

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irradiation (Antoccia et al., 1994; Vral et al.,

Genomic instability.

There is now substantial evidence that genomic instability in the form of chromosome aberrations carry over to several cell generations after irradiation is influenced by radiation quality. When either γ - or neutronirradiated human epithelial cells were examined for CA between 5-40 population doublings, neutron-irradiated cells showed consistently elevated frequencies of aberrations, while the frequency for γ was not significantly different from control until 20-35 doublings (Ponnaiya et al., 1997). The radioresponse of those cells carrying genetic instability can be different from

Genetic disorder & radioresponse of neutron.

Since, low-energy neutron-induced DNA lesions are less repaired, an observable differences in cytogenetic response may not be possible between exposed persons of average capacity and with inherent repair deficiency. Hence, the investigation of the health effects of radiation requires the most accurate estimation of absorbed dose by the individual or estimation of the radiobiological risk. Generally, patients with genetically inherited disease ataxia telangiectasia (AT) display a reduced relative sensitivity to radiation of increasing LET. After neutron irradiation, the increase in MN yield above control was less pronounced in AT lymphocytes compared with X-rays or gamma

Fig. 6. CA induced by monoenergetic neutrons (Gajendiran et al., 2000; Tanaka et al., 1999)





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1996). This differential response can influence the outcome of RBE value in these cells. A suitable risk assessment is needed for such persons carrying known genetic disorders.

Adaptive factor in modifying neutron insults.

Radiation induced risk is modified an extent by adaptive response of an organism as a result of its previous exposure to low-LET radiation. Either a faster repair kinetics or reduced initial damage was found evident in adapted lymphocytes (Wojcik *et al.*, 1995). This induced-DNA repair mechanism may able to heal genetic damage certain level. This is quite evident in a study conducted on flight engineers

Fig.7. Adaptive response of human lymphocytes towards ionizing radiation (Gajendiran et al., 2001)



*Represents the mean of each experimental group obtained by subtracting the control value from those experimental values that happen to fall at the maximum over the values of the control group (which equals to the mean plus 95% confidence limit). Error bar represents (±) SD of the mean

that were exposed to cosmic neutron (Zwingmann et al., 1998). In this study it was shown that higher oxidative DNA damage was noticed in flight engineers with a relatively short exposure history (mean cumulative exposures 30.7±1.7mSv) than the group with a longer history and consequently relatively higher cumulative radiation doses (mean cumulative exposure 53.6±3.1 mSv). In the latter case, the oxidative DNA damage had returned to base line levels, which was paralleled by an induction of DNA repair activity, indicating adaptation to long-term exposure to cosmic radiation. Marple and Skov (1995) observed that 0.2 Gy of d(4)-Be neutrons could produce an adaptative resistance to subsequent 1 Gy challenge doses of X-rays and the adaptation was at least as large as that induced by 0.2 Gy X-ray dose. A recent study conducted by us (Gajendiran et al., 2001) with human peripheral lymphocyte supports the neutron induced

adaptive response. A prior exposure to 0.0025 Gy californium neutrons nullified the excess tail moment caused by 0.25 Gy given after 4 h gap. Same way, prior exposure to 0.01 Gy ¹³⁷Cs- γ can offer cross-adaptive response to further neutron exposure (Fig.7). The *in vitro* radioresponse of A-bomb survivors was also different from the unexposed group. The radio-adaptive response has greater role in tumour treatment and also account for discrepancies in the observed biological responses.

Biological effect of cosmic neutrons

Colonization of Moon and Mars may take highest priority in the coming millennium. Cosmic radiation creates high-energy secondary radiation consisting mainly of neutrons, protons, mesons and γ -radiation. The historic Apollo XI mission to Moon, provided about information astronauts' radiation exposure. The total mission dose equivalent of 4.02 mSv for a mission of 195.3 h duration was measured from the nuclear emulsions and plastic foil detectors carried by the astronauts. The exposure was mainly from nuclear particles (Schaefer et al., 1972). The frequency of dicentrics found in lymphocytes of MIR cosmonauts before and after the last space flight compare well with frequencies expected from doses of low- and high-LET radiation to which they were exposed during the voyage. These data, combined with estimates of radiation exposure during 975 flight days to Mars, Obe et al. (1999) predicted a radiation

hazard of 40 dicentrics expected to occur in every 1000 cells of T lymphocytes, at solar minimum period, even in the presence of 20 g/cm² Al shielding.

The introduction of commercial flights at high altitude could result in more individuals being exposed to neutrons than terrestrial workers, emphasizing the in necessity for better-defined risk estimate (Pandita & Geard, 1996). At equal cruising altitude (10-18 km) of the commercial flight, the secondary radiation forms the major part of the dose received by the aircraft users. The potential radiation risks to the high altitude flight users have been examined by NCRP (1995) and also recognized the need for more information about the radiobiology of neutrons. The cosmic ray equivalent dose rates (with neutrons as the largest single contributor at higher altitudes) estimated to varying from 5-30 µSv/h depends upon the altitude (Pandita & Geard, 1996). In another study, the dose equivalent during commercial high altitude flight is estimated to be about 6 μ Sv/h and it can reach up to 1 mSv/h for a brief period of solar flares (NCRP, 1995). Increase of chromosomal aberration in peripheral lymphocyte and elevated incidents of cancer among aircrew

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been well documented members have (Romano et al., 1997; Zwingmann, 1998). From the results of epidemiological studies on flight personnel, Kuni (1998) derived a radiationweighting factor of 75 for low dose and lowdose rate neutrons. Heimers (1999) assigned RBE value for simulated cosmic radiation up to 64 for the induction of dicentric chromosomes and up to 113 when calculating only the high chromosomal component. LET Since aberrations are correlated in enhanced cancer risk, biological response takes an important position in mission planning and safe flying.

5. Cyotgenetic fingerprint of neutron exposure

The concept of neutron fingerprint stems from the fact that the proximity effects of DNA lesion play a vital role in the type of aberrations and the pattern of formation may be related to the radiation type. Fast neutrons of 1 MeV were found more efficient at inducing insertions compared to X-ray 200 keV and was suggested that the relative frequency of insertions could be used as a "fingerprint" for exposure to high LET radiation (Grigorova et al., 1998). Increased acentric rings were also identified with the exposure of 252 Cf neutrons filtered through 15-cm iron block (Tanaka et Same way, different types of *al.*, 1994). chromosomal aberrations can result from the interaction and illegitimate reunion of reactive double strand breaks (dsb) within the same chromosome (intra-chromosomal exchange) or different between chromosomes (interchromosome exchange) and their relative frequency may be the indicative of the type of radiation involved. Most experimental human data is based on the measurement of the ratio of inter-chromosomal (dicentrics) to intrachromosomal (rings) unstable exchange type aberrations (Fig. 3). This ratio designated as F by Hlatkys et al. (1992). Later, Brenner and Sachs (1994) considered the ratio of interchromosomal to intra-chromosomal, interarm aberrations, either for stable aberrations (translocations to pericentric inversions) or for unstable aberrations (dicentrics to centric rings). Since stable aberrations can be measured many years after exposures, the F value measurement was seen as practical biomarker of past exposure to densely ionizing radiation. At equal low doses, densely ionizing produce radiation such as neutrons chromosome breaks that are closer together making multiple breaks within a single chromosome comparably more frequent than yray. Consequently, yields of intra-chromosomal aberrations after exposure to low doses of densely ionizing radiation are increased even further relative to inter-chromosomal aberrations, and the resulting smaller F value is then a "fingerprint" of a past exposure.

According to Brenner (1996), neutron produces significantly smaller F value of ~6 compared to 15 for X- or γ -rays. It leads to the conclusion that F value is significant to act as genuine fingerprint of neutron exposure. Based on the data analysis on the F value in A-bomb survivors, it was concluded that majority of the biological damage at Hiroshima was probably caused by neutrons, not photons. At the sametime, critiques also pointed out the insufficient experimental data to support the concept of F ratio. They also emphasized the F value 5.2 for X-ray was not different from the value for neutron (Bauchinger & Schmid, 1997). Thus LET dependence of F ratio has raised doubts. Yet, Lucas (1998) proposed "S"-value to understand the "signature". Accordingly, the ratio of induced complete translocation to incomplete translocations as seen by FISH, can be a cytogenetic fingerprint of exposure to radiation of different quality. It takes the advantage that incomplete translocation remain over 30 years, at least in monkeys. A physical explanation for the proposed correlation with LET was, at high LET radiation the chance of producing an incomplete chromosome exchange increases with the total population of exchange as the densely packed dsb likely to lead incorrect and incomplete rejoining. Also, high LET produces more smaller chromosome pieces compared to low LET, which could amount for an increase in terminal pieces too small for a translocation to be detected. The small translocations would appear as an incomplete. The S ratio was 2 for densely ionizing radiation in contrast to a value of 10 for sparsely ionizing radiation. The data of A-bomb survivors was shown in S value of 3.25, suggesting significant neutron component in Abomb radiation exposure. However, Nakano et al. (1999) obtained the S value of 3.16 for Xrays which differ from 10 reported previously, and not larger than the 3.25 obtained from the blood lymphocytes of A-bomb survivors. Thus, S values seem to vary among laboratories even after exposure of cells to sparsely ionizing radiation. Meanwhile, more reliable approach in the "fingerprint" was postulated as G-ratio (Sachs et al., 1997), in which a clear LET dependence for the yield ratios of intra-arm interchanges to inter-arm intrachanges (interstitial deletions/ centric rings (R_c)= G ratio, where interstitial deletions= acentric rings (R_0) Min) was shown. The increase in + effectiveness was about 3 fold for the densely ionising particles compared to that of sparsely ionizing γ -rays. The other alternate approach Hratio (interstitial deletions/dicentrics) proposed by Bauchinger and Schmid (1998) also seems to be potential indicator model. Thus, cytogenetic end points can serve as signature



of the past exposure especially for low energy neutrons.

6 Discussion

The radiobiology of neutron gains importance amidst the prevailing nuclear arsenal (Doty, 1999) (including H-bomb), nuclear power plants and neutron therapy facilities. Since 1945, more than 2,050 nuclear tests were conducted in which 26% of them were in atmosphere (Norris & Arkin, 1998). As mankind steps into the new millennium especially with the ambition of exploring the cosmic world, the type of neutron source and the exposure pattern can also be different. Therefore, the risk assessment for neutron exposure requires RBE value for different energies at different condition.

Table 3. Average yield of genetic damage/ Gy/mammalian cell (Nikjoo et al., 1998)

Radiation Effect		High FT
Tracks in nucleus	1,000	2
Ionization in	1,00,000	1,00,000
nucleus		
Ionization in DNA	1,500	1,500
Base damage	10 ⁴	10 ⁴
RBE of dsb	~1	~1
PCC break: initial	6	12
: 8 h	<1	4
CA	0.3	2.5
Dicentrics	0.1	0.8
Complex	10%	45%
aberrations		
HPRT mutations	10 ⁻⁶	10 ⁻⁵
Lethal lesions	0.5	2.6
Cell inactivation	30%	85%

The variation in neutron RBE reflects the complex function of neutron dose and its intricacy in biological processing. A range of neutron RBEs need to be considered in formulating guidelines for those who work in energy facilities, airline crewmembers, and transoceanic polar voyages and in neutron therapy. Low energy neutrons are the principal concern in radiation protection. They have little or no (dose)² component and display a linear response curve. These neutrons effectively damage cells at hypoxy condition, which has potential role in tumour treatment. Owing to high biological response, the role of neutron in A-bomb exposure at Hiroshima, cannot be neglected altogether. The RBE_M values for hiroshima-type neutrons (>0.2 to <2 MeV) are among the highest reported for cytogenetic RBE increases, generally, with effect. decreasing neutron energy (Van Dam et al., 1992). The peak biological effect is observed at a narrow window range of >220 keV to <430 keV. The physical property of the contributing energy groups of neutron beam is essential to

particular bring about changes in macromolecule, DNA. For 230 keV monoenergetic neutrons about 50% of recoil protons will travel ~1.3-2.7 µm, depositing energy at a consistent mean of about 80 keV μ^{-1} . Those protons of remaining 50% travel <1.3 µm have a declining mean LET as range and energy decrease. In contrast, about 50% of the recoil protons from 1910 keV neutrons travel about 20 μ m or more with mean LET <50 keV μ m⁻¹. Whereas, the lower energy neutrons (230, 320, 430 keV) all produce recoil protons with ranges <6 µm (lesser than cell nuclear radii) where the majority of recoil protons have mean LET greater than 80 keV µm⁻¹ (Geard, 1996) and hence exhibit the peak biological effect.

 Table 4. Cytogenetic fingerprint ratio for

 neutrons and other high LET radiation

Interstitial deletion/	"G " ratio (Sachs et al.,
centric rings	1997)
Interstitial deletion/	"H" ratio (Bauchinger &
dicentrics	Schmid, 1998)
Dicentrics/ centric	"F" value (Hlatkys <i>et a</i> l.,
ring	1992; Brenner & Sachs,
	1994)
Complete-/	"S " ratio (Lucas, 1998)
incomplete-	
translocation	

Aberrations and energy deposition events (recoil protons) are linearly related. The microdosimetry of monoenergetic neutron is thus important for the outcome of biological response. Neutron induced genetic insult is considered to be more severe than low LET radiation. This is mainly because of lesion complexity rather than difference in the number of initial damage they sustain. Low energy neutrons are effective in producing LMDS, which can be more mutagenic or carcinogenic when compared to less densely ionizing radiation. The severity of the neutron induced lesions are effectively amplified in increasing resonance with the growing complexity of the endpoints such as simple dsb, mutation frequency, chromosomal aberrations, cancer and mortality (Table 3).

The proximity and complexity of neutron induced DNA lesion lead to type of genetic aberration, which could serve as fingerprint. The various ratios suggested for neutrons as fingerprint (Table 4) are essentially based on the cytogenetic end points. It is expected in neutrons, in view of the dense ionization tracks generated, interchanges (interstitial deletions, rings, pericentric inversions) between the arms will be favoured over interchanges between chromosomes (dicentrics and translocations). A biomarker that would distinguish neutron induced biological damage from that of other agents or



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from mixed radiation has long been the goal in 4. radiation biology. Deeper understandings of RBE of low energy neutrons and the cytogenetic fingerprint ratios will be useful to achieve the goal. A recent criticality incident (Clark, 1999) only emphasizes the need to 5. adapt suitable biodosimetry method to evaluate the neutron dose rapidly especially in a acute and mixed exposure condition. It will help to render appropriate medical help to the exposed victim. 6.

Radioadaptive response can be an influential factor in modifying the outcome of neutron exposure. It is evident that biological dosimetry resulting from high-neutron-dose could be modified by a prior exposure to low doses of low-LET radiation or neutron. Hence, long-term neutron exposure at low levels needs deeper study. The health of flight personnel and astronauts may be of greater concern in future. In the current high altitude commercial flights. both passenger and crew are subjected to very low doses of neutrons. About 500 million people crosses transcontinental border every year. The possibility of more people having exposed will be high in the coming millennium. The human genome project and the modern 9. powerful computers can serve as essential tool for identifying genes and chromosomal targets as primary steps for understanding the mechanism involved in radiation risk.

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