Cytogenetic Effects of Tritiated Water (HTO) in Human Peripheral Blood Lymphocytes *in vitro*

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ABSTRACT The yield and distribution of unstable chromosome aberrations induced in human lymphocytes by tritiated water (HTO) has been measured. Tritiated water was mixed with heparinised blood in calculated amounts so as to give 0.1 Gy to 1.5 Gy, 30min and 2 hours. After culturing for 48 hrs, the dicentric yield was measured as a function of dose to the blood. Using a linear quadratic dose-effect relation to fit the experimental data, a significant linear contribution was found. The α, β values were found to be $8.25 + 0.4 \times 10^{-2}$ Gy^{-1} and $6.4 + 0.2 \times 10^{-2}$ Gy^{-2} respectively. Micronuclei yield at low doses could be fitted to a linear equation $Y = C + \alpha D$ and indicates a á coefficient of 0.172 ± 0.003. This value is found to be 2.18 and 2.8 times higher than those previously reported for X and gamma rays respectively. Hence β- rays are found to be more efficient in producing two lesions with single ionizing tracks at low doses.

INTRODUCTION

Tritium is a radionuclide of low toxicity and, has wide application in research. It is also produced as a by product in Nuclear Industry (Rower and Wilcox 1967). Annually mega curies of tritium is being released into the environment and it gets converted into tritiated water (HTO). Tritiated water can be ingested in the liquid form or it can be inhaled or absorbed through the skin in the form of water vapour. This makes tritium an occupational hazard in CANDU type nuclear reactors. Once it gets incorporated into the water compartments of the cells HTO causes an internal exposure to low energy β particles with a maximum energy of 18 keV and an average energy of 5.7 keV. In the biological system, a fraction of HTO also gets incorporated into the DNA of cells and RBE values of 2-3 were also reported relative to low dose rate gamma rays in germ cells of different animals (Commerfield et al. 1977). The tritiated water is metabolized like ordinary water and has a biological half-life of 10 days in man and exposure is considered as chronic in nature (Butler and Roy1965). Present studies are aimed at developing method of assessing absorbed radiation dose.

Chromosome aberration dosimetry is the most reliable technique for the assessment of radiation accidents involving acute external radiation exposures (Bender and Gooch 1966; Evans 1962,

1977; Brewen et al. 1973; Lloyd et al.1978; Ramalho et al.1988; Sreedevi et al.1993; Hayata et al. 2001). The technique has limited application in cases of accidental intake of radio nuclide due to inhomogeneous exposure resulting from the concentration of material in various target organs, its retention time, route of entry, physico-chemical form, quality of the radio nuclide and its metabolism. An exception to this is the isotopes of Cs, which concentrates in muscles and tritiated water, which equilibrates with the body water and produces a uniform irradiation (Lambert et al. 1971; IAEA 1986). An industrial accident involving the incorporation of 35 GBq was treated by forced diuresis. The cytogenetic dose estimate of 0.4 Gy by Lloyd et al. (1986) was in good agreement with the effective dose equivalent of 0.45 Sv calculated by measuring the concentration and excretion of tritium in urine. Follow up studies were conducted on the same case using FISH translocation assay and the retrospective dose estimate was in agreement with the initial dose estimate by dicentric analysis (Lloyd et al.1998) .

Micronuclei analysis in cytokinesis blocked peripheral blood lymphocytes has been suggested as an alternative to the scoring of dicentrics in case of radiation accidents. Muller and Streffer (1994) have reviewed the effect of various radiation qualities and dose rates on the induction of micronucleus. Micronuclei arise from

chromosome fragments or whole chromosomes lagging behind   the genome at cell division and appear as a small mass of DNA adjacent to the main nucleus. Lymphocytes blocked in their second interphase by the addition of cytochalsin-B (Cyt-B) appear as binucleated and micronuclei are scored in these cells stained with Giemsa or fluorescent dyes (Fenech 1993). Individual variability in the dose response and unknown background frequency limits its application in the low dose region (Sreedevi and Rao 1994).

Significant differences between laboratories exists in the *in vitro* dose response parameters and the interpretation of dose using calibration produced elsewhere may introduce uncertainty in the dose estimate and therefore IAEA (1986, 2001) recommended that any laboratory intending to carry out biological dosimetry should establish its own dose response curve. Hence the present studies are aimed at obtaining in vitro dose response data for the induction of chromosomal aberrations using tritium beta rays. Micronuclei induction at low doses was also measured and it is compared   with the dose response data previously obtained for X-ray and gamma rays (Sreedevi and Rao 1994; Sreedevi 1994).

MATERIAL AND METHODS

Irradiation with Tritium Beta Rays: Blood samples were obtained from 3 healthy donors by vene-puncture and irradiation was performed by adding increasing amounts of HTO to 1 ml samples of whole blood contained in 20 ml glass tubes. Exposure times were 30 min in the dose range of 0.1 Gy to 0.4 Gy and 2 hours in the dose range of 0.9 Gy and 1.5 Gy. Washing the cells three times with the culture medium terminated the irradiation with tritium. Each time the cells were re-suspended to 20 ml with fresh washing fluid and centrifuged at 2000 rpm for 5 min and the supernatant was discarded. Following irradiation, the samples were made up to initial 1 ml by adding fresh medium supplemented with serum as reported by Vulpis (1983). It is assumed that all the tritium is washed in the post treatment since residual activity in the cells was found to be less than 0.2% by liquid scintillation counting. The Tritium β dose was calculated as follows: -

Activity (Bq) x Av. Energy (MeV) x Energy (ergs) x Duration of Irradiation (min) x water content

Hence the dose rate for an activity 0f 1 M Bq/mL is

$1x\,10^9\,(5.67\times10^{-3}\,\text{Mev})\,(1.6\times10^{-6}\text{erg})\,(0.8\,x60)/\,100\,x\,100 = 43.8\,\text{mGy/min/MBq}$

A small additional dose due to the residual activity was unavoidable and hence ignored

Culturing and Slide Preparation: Whole blood lymphocyte cultures were set up from each irradiated sample by mixing 0.5 mL of blood with Ham's F-10 medium supplemented with 15% fetal calf serum and 0.1 mL of PHA (10μgm/ml) and BrdUrd (final concentration in culture 10μgm/ml) for 48 hours at 37^0 C in the dark (Bhatt et al.1984; Sreedevi and Rao1998). About 0.1 mL of 5 μgm/mL of colcemid was added 3 hours prior to harvesting. Cells were treated with 8mL of KCl (75 mM) at 37^0 C for 15 minutes, then fixed with freshly prepared Methanol: Glacial Acetic acid (3:1, v/v). After three washes in fixative cell suspension was dispensed in to slides. The chromosome preparations were stained by the modified method of Perry and Wolff (1974). Finally the slides were stained with 4% Giemsa for 10 minutes, air dried and mounted in DPX.

Micronuclei cultures were incubated at 37^0C for 72 h. Cytochalasin B (Sigma, St.Louis, Mo) was added at 44 h to have a final concentration of 5 μgm/mL to block the cells in cytokinesis. At the end of 72 h incubation, cells were collected, treated with 0.75 M KCl for 2 min, fixed in 4:1 methanol acetic acid mixture. All the slides were coded and stained with Giemsa.

Analysis of the Slides: About 500 first cycle metaphases having uniform staining and which contained 46 centromeres were analysed for the presence of unstable aberrations such as dicentrics, rings and acentrics. At higher doses scoring was restricted to 100 dicentrics per dose point. For micronuclei induction a total of 1000 binucleated cells with well-preserved cytoplasm and cell membrane were analysed for the presence of micronuclei under a magnification of 650x using a modified criteria of Heddle (1973).

RESULTS AND DISCUSSION

The yield and distribution of unstable dicentric and ring aberrations (pooled data from three donors) are presented in Table1. The mean number of observed dicentrics per cell (Y) is given for each dose (D) along with relative variance (σ^2/Y) and the dispersion index (u). The value of u varies from - 0.114 to 0.67 . In all the dose points the value of u is less than 1.96 indicating a Poisson distribution. This is in

consistent with the observations of the distribution of dicentrics by X and Gamma radiation (Edwards et al. 1979; Sreedevi 1988). The presence of few chromatid aberrations in the irradiated lymphocytes confirms the fact that the amount of activity left in the cells is negligible (data not shown). Figure 1 shows the fitted dose response expression

$Y = 8.23 \pm 0.04D + 6.4 \pm 0.2D^2$ for the induction of dicentrics along with other acute dose response established for X-rays, γ rays and neutrons (Seedevi et al.1997; Sreedevi 1998). The values of α, and β coefficients are given in Table 2. The a coefficient for β-rays was found to be higher than that for X-rays and γ rays. The β coefficient does not show much marked difference. A property of the linear quadratic function used is that the quotient α / β is the dose at which the number of aberrations produced by single and two ionizing track events are equal.
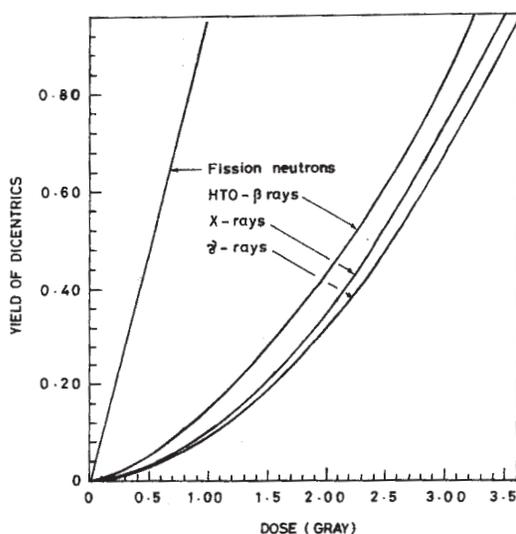

Fig. 1. Calibration curves for the induction of dicentrics.

Below this dose majority of the aberration will be produced by single track (IAEA 1986). For tritium β-rays α / β is 1.29 Gy and for X and γ it is 0.42 Gy and 0.48 Gy respectively. Higher a value for tritium reflects the differences in the LET of the three radiation types. The electron tracks produced by β particle radiation are more efficient at producing two lesions with a single ionizing track than the electron tracks produced by X, γ radiation. The yield of MN induced by various doses of β-rays for three blood donors is given in Table 3. For one donor 0.2 Gy data is not available due to culture failure. The dose response fitted to a linear model represented by

$Y = C + \alpha D$ where Y is the frequency of micronuclei corresponding to a dose D in Gy and α is the linear coefficient. The yield equation is as follows:

$Y = 0.0086 \pm 0.0068 + 0.17 \pm 0.003$

In a previous study by Sreedevi and Rao (1994) the corresponding α coefficients for X-rays and g rays are 0.08 and 0.06 respectively. Figure 2 shows the dose response along with X and gamma response at low doses. At low doses the limiting RBE_2 is obtained by comparing the coefficients for X-rays, γ rays and is found to be 2.12 and 2.8 respectively. Hence β rays are found to be more efficient in producing two lesions with a single ionizing track at low doses. Ueno et al. (1982) has reported an RBE of 1.8 to 2.3 at low doses for micronuclei induction at a frequency of 50 or 25 micronuclei per 1000 cells; 1.8 at all doses for the induction of resistance to 6-thioguanine; 1.5 for 50% survival for cell killing, in L51785 mouse lymphocytic leukemia cells. In another study using blood lymphocytes Mill et al. (1996) reported a low dose limiting RBE of 0.5 for beta particles. The variable background frequency of micronuclei limits its application as a biodosimeter at low doses. In the absence of a pre-exposed control value CB MN assay can only

Table 1: The observed distribution and yields for dicentrics production in lymphocytes irradiated with HTO β-rays

Average dose (Gy)	Total cells scored	Dicentrics + centric rings	Dic + rings/ cell (Y)	Dicentric distributions				u	σ^2/Y
				0	1	2	3		
0	1000	-	-	-	-	-	-	-	-
0.1	1203	5	0.004	1198	5	-	-	-0.114	0.998 ± 0.02
0.225	1050	17	.016	1033	17	-	-	-0.386	0.986 ± 0.02
0.375	1002	23	0.023	979	23	-	-	-0.58	0.978 ± 0.044
0.90	450	49	0.109	403	45	2	-	-0.41	0.977 ± 0.01
1.50	250	120	0.48	159	65	23	3	+0.67	1.06 ± 0.12
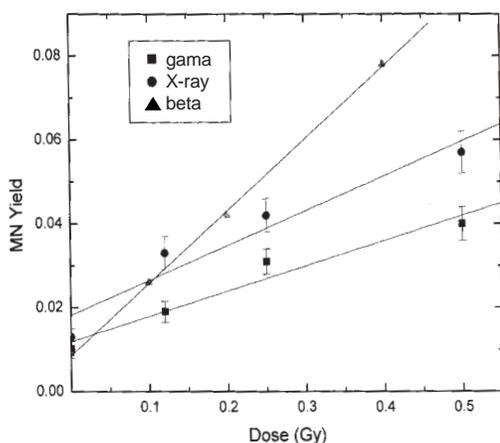
Fig. 2. Dose response curve for the induction of dicentrics +rings beta rays (▲), X rays (●),and gamma rays(■)

pan-centromeric probe or antikinetochore antibody may possibly reduce the variability in MN frequency by avoiding the detection of centromere positive MN. In Istanbul accident MN assay was used along with dicentric and translocation assay for dose assessment. The dose estimation by MN assay agreed well with the dicentric assay (IAEA 2000). Fully automatic analysis of MN by image analysis and pattern recognition procedures or by flow cytometry may be helpful for its potential application in a mass screening role for rapid biodosimetry of large number of people during a radiation emergency (Tates et al. 1990; Schriber et al. 1992) .

The low dose limiting RBE ratio of tritium β-rays to X-rays for dicentric production is 2.34. Since there are many factors, which influence RBE, it is difficult to compare values of RBE obtained by different investigators employing

Table 2: Values for coefficients α and β in linear quadratic dose response relationship ($Y = \alpha D + \beta D^2$) for Dicentrics + centric rings

Dose rate Gy/min	Dose range (Gy)	Type of radiation	$\alpha \pm SE\ 10^2 Gy^{-1}$	$\beta \pm 10^{-2} Gy^{-2}$	$\alpha / \beta\ (Gy)$
0.5	0.12- 6	Co-60 γ rays	2.7 ± 0.5	6.5 ± 0.7	0.42
1	0.1 - 2	X-rays	3.3 ± 0.7	6.8 ± 0.3	0.48
-	0.1 - 1.5	HTO – β rays	8.23 ± 0.4	6.4 ± 0.2	1.29

Table 3: The observed distribution and yields for micronuclei (MN) production when lymphocytes are irradiated with ^{3}H β-rays.

Average dose (Gy) Code No.	Total cells scored	Total MN observed	Distribution of MN				MN/Cell
			0	1	2	3	
0.1 Gy							
TRB	1000	22		22			0.022
TRS	1000	32		27	1	1	0.032
TRP	1000	25		23	1		0.025
Total	3000	79		72	2	1	0.026
0.2 Gy							
TRS	1000	46		35	4	1	0.046
TRP	1000	37		31	3		0.037
Total	2000	83		66	7	1	0.042
0.4 Gy							
TRB	1000	84		71	5	1	0.084
TRS	1000	70		56	7		0.07
TRP	1000	80		56	9	2	0.08
Total	3000	234		183	21	3	0.078
C1	1000	8		8			0.008
C2	1000	10		10			0.01
C3	1000	11		9	1		0.011
Total	3000	29		27	1		0.009

detect exposures in excess of 0.25Gy for X and γ rays (Sreedevi and Rao 1994). But for β-rays the α coefficient being higher there is a possibility of detecting a low dose of 0.15 Gy. The use of

different endpoints and experimental conditions. Present results show that HTO- β rays are more effective than X-rays especially at low doses. The studies conducted by Vulpis (1984) revealed

an RBE of 2.6 for HTO-β rays as compared to X-rays in the low dose region. The values obtained in the present studies show considerable agreement with those reported by Vulpis. Other values reported in literature are 1.17 by Bocian et al. (1977) and 1.13 by Prosser et al. (1983). The differences obtained can be attributed to the differences in the level of the effect chosen for comparison, techniques and procedures like incubation time, range of concentration of tritium, irradiation of the stimulated cells rather than the whole blood which is known to give higher aberration yield particularly at low doses. Other factors are the dose rate at which the reference radiation was delivered and the dosimetry of β-rays.

Since tritiated water has an effective half – life of 10 - 12 days, in the event of an accident the exposure will be protracted and the dose estimate can be made from the linear relationship $Y = \alpha D$ and the β term is disregarded. An *in vivo* study in power plant workers exposed to both external gamma and internal tritium and having less per capita dose revealed a higher yield of chromosomal aberrations compared to age, sex matched externally exposed power plant workers (George et al. 1983). This may be attributed to the higher RBE of tritium at low doses. More studies are warranted to understand the combined effect of external and internal exposures.

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