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EFFECTS OF TRITIATED WATER INGESTION ON MICE: II. DAMAGE AT CELLULAR VIS-A-VIS SUBCELLULAR LEVEL MONITORED UPTO FOUR GENERATIONS

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INTRODUCTION

Practically entire man-made tritium (^3H) produced is directly released to the environment, mainly in form of tritiated water (HTO). This may cause a low-dose, chronic exposure to the population especially those inhabiting close to the source of release of HTO i.e. heavy water reactors, fuel reprocessing plants etc. Jacobs (1968) estimated that such population may receive doses like 40 to 110 μSv per year while recommended MPD is 500 μSv. Since the use of nuclear power as source of energy is imminent it is expected that by the advent of next century production of man-made tritium may be two to three times greater. Further, in the advent of fusion technology in the coming decades the problem may become more severe. Commerford and co-workers (1977) have recommended that humans could be exposed to tritium levels as high as 3.7 kBq/ml in their drinking water. This recommendation is based on the assumptions that the MPD is 50 mSv/year and the RBE of tritium is 1. This is obviously wrong since the MPD of 50 mSv/year is for radiation workers and not for general public for whom the MPD is 5 mSv/year. Further it has already been accepted that the RBE has to be more than 2 (Okada and Nakamura, 1980; Feinendegen, 1979) although the dual radiation action theory (Rossi, 1977; Kellerer and Rossi, 1971, 1972, 1978) predicts that RBE may be as high as 4. Microdosimetric studies of Ellett and Braby (1972) have suggested a RBE of 3.7. All these demand a thorough investigation into the biological effects of low-dose chronic irradiation by HTO for the purpose of radiation safety. Earlier damage was assessed at either cellular level (Srivastava, 1978; Kapoor and Srivastava, 1979; Carr and Nelon, 1979; Srivastava, Bhatia and Kapoor, 1979) or subcellular levels (Zamenhof and Van Marthens, 1979, 1981; Sharan and Srivastava, 1980; Commerford, Carsten and Cronkite, 1977; Dobson and Kwan, 1976). In the present report, a different approach has been taken to assess direct damage at cellular level vis-a-vis subcellular level for proper understanding of radiation effects.

Damage at cellular level is measured using colony forming units in spleen (CFU-S) technique while that at subcellular level by DNA unwinding technique. The damage was monitored upto four generations in Swiss albino mice.

MATERIAL AND METHODS

Mode of Administration of HTO

A group of 4-week-old mice were given tritiated drinking water at a dose rate of 37 kBq/ml (Bhabha Atomic Research Centre, India) and standard mouse pellet (Hindustan Lever, India) and were bred to raise generations by litter-mating in our own animal house (not germ free). All successive generations were maintained on the same HTO. 6-7 week-old female mice from each generation served as donors of bone marrow cells (BMC).

Colony forming Units in Spleen Technique

The technique followed is described elsewhere (Till and Mc Culloch, 1961). The recipient 10-12 week-old males were irradiated (7.95±0.05 Gy per animal) by a ^{60}Co source (dose rate = 0.2936 Gy/sec) four hours before BMC injection. 5×10^4 BMC in 0.2 ml of MEM (without bicarbonate) (Centron Agro Industries, India) was injected into the heat dialated caudal vein of recipients. Control animals received BMC from animal unexposed to HTO while another group of animals received only 0.2 ml MEM to serve as base-line. After injection animals were housed in the same animal house. Survivors were killed on the ninth day, spleens removed in Bouin's fixative, and superficial colonies were counted.

DNA Unwinding Technique

The method followed was same as described by Rydberg (1975) with minor modifications. 6.25×10^6 BMC in 0.5 ml of PBS (pH 7.2) (CSIR Centre for Biochemicals, India) was forcefully emptied into 1.0 ml of alkaline solution (pH = 11.6 and I = 0.98) and incubated for 60 min. in dark at room temperature. It was neutralized by 1.0 ml of HCl (0.034 M), sonicated for 60 sec at 12 μ amplitude by exponential probe in MSE Ultrasonicator and finally SDS was added to get final concentration of 0.4%. Five columns (0.7 cm x 4.0 cm; volume 1.5 ml from Bio Rad, USA) were packed simultaneously with Hydroxyapatite DNA grade Bio-Gel HTP (Bio Rad, USA) and equilibrated at 62°±2°C in an oven. A hydrostatic pressure of 20 cm column height was applied and finally single strand DNA (ss DNA) and double strand DNA (ds DNA) were collected in 3.0 ml each of 125 mM and 250 mM Potassium phosphate buffer (pH 6.8-6.9) respectively. Total, ss- and ds DNA contents were determined by diphenylamine method (Burton, 1968) on Shimadzu Recording Spectrophotometer UV-240 (600 nm).

RESULTS AND DISCUSSION

The results show drastically reduced colony forming ability in F_0 mice BMC which recovers a little in F_1 and acquires a steady state level till F_3 generation (see Table 1). On plotting survival fraction (percent of control) for BMC against generations of mice, the plateau is found around 50% survival (see Fig. 1). This clearly indicates that chronic exposure to HTO at low-dose (the total dose was calculated according to Dobson and Cooper (1974) and Brookes and co-workers (1976) and was estimated to be $>$ 43 mGy for F_0 animals and $>$ 92 mGy for other generations) has a tendency to recover or acclimatize to certain extent. However, the damage to survival is not fully eliminated and is carried through the succeeding generations.

TABLE 1

Donor Group	# of Animals/ set(Survivors on Ninth Day)	# of Colonies per Spleen*	CFU/10^4BMC*	Survival Fraction*
Base line	13 (2)	0	-	-
Control	16 (5)	15.40±1.03	3.08±0.20	1.00±0.06
F_0	15 (7)	4.83±0.74	0.96±0.15	0.31±0.05@
F_1	15 (6)	7.50±1.08	1.50±0.12	0.49± 0.04£
F_2	07 (5)	7.00±1.03	1.40±0.12	0.45± 0.04
F_3	10 (2)	8.00±1.00	1.60±0.14	0.52±0.06

*Mean ± SEM; @ Significant as compared to F_1, F_2, and F_3; and £ Not significantly different from F_2 and F_3.

It is proposed, at this point, to test the role of DNA in colony forming ability of BMC. The result is shown in Fig. 2 below.

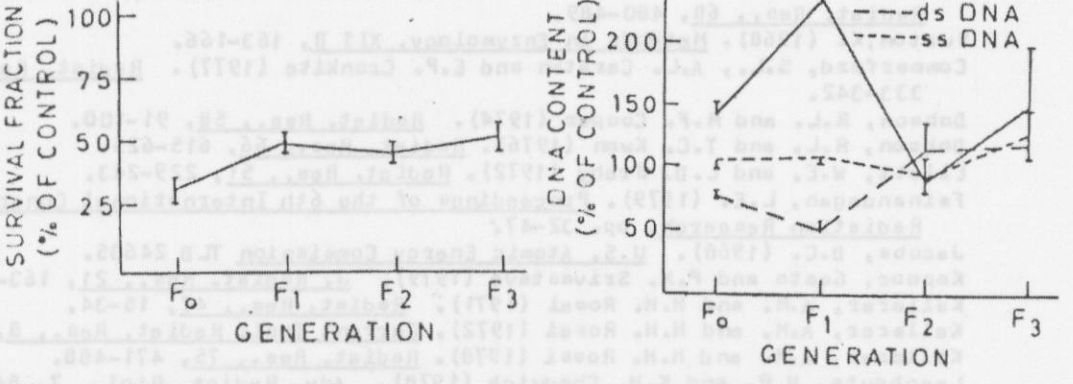

Fig. 1. BMC Survival fraction against generations of mice as revealed by CFU-S technique.

Fig. 2. Contents of DNA as calculated by DNA unwinding test and plotted against generations of mice.

It shows minimum damage to DNA in F_0 which goes on increasing upto F_1 unlike survival (fig. 1) where damage to BMC is maximum at F_0 and survival or colony forming ability increases in F_1. However, later from F_1 through F_3 damage to DNA partially correspond to survival or colony forming ability. This clearly indicates that, at least, initial impairment of cloning ability is not DNA dependent but related to some other factor(s). Moreover, strand breaks in DNA by ^3H can be caused only by transmutation effect. By F_1 generation enough ^3H is likely to be incorporated to cause strand break by transmutation and hence increased damage to DNA (Fig. 2) from F_0 to F_1. The result shows repair of DNA damage to a greater extent but survival remains essentially about 50%. Since there is no report to directly correlate DNA strand breaks with eukaryotic cell survival (Leenhouts and Chadwick, 1978) it seems reasonable to propose that HTU is initially effecting factors like enzymes and/or membranes to cause reduction in homing ability of BMC. Also,

cloning or homing of exogenous BMC in host spleen is primarily a phenomenon related to membrane or membrane-bound factors. Since the survival remains suppressed upto F_3 while damage at DNA level, especially ds DNA, recovers sufficiently it is likely that at this low dose of irradiation DNA is not the primary target. After an intial recovery from F_0 to F_1 the survival fraction essentially remains approximately 50% below control till the end of experiment which may be grouped as irrepairable damage to BMC. Few questions which arise now are (i) What is the target which is getting damaged at this low-dose of radiation? (ii) Can there be any radiation dose which may be called 'safe'?

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