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Radiotoxicity of Chronic Ingestion of Tritiated Water on Liver HMG Proteins of Swiss Albino Mice

R N SHARAN, R SCHAFFRATH[1], LALTANPUIA and F H A SCHNEEWEISS[2]

Radiation & Molecular Biology Unit, Department of Biochemistry, North-Eastern Hill University, Umshing, Shillong 793 022

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Low-dose exposure for one generation of Swiss albino mice to tritium administered by chronic ingestion of tritiated drinking water (HTO; 37 kBq ml^{-1}) was found to be radiotoxic to the high mobility group (HMG) proteins of liver (cumulative dose: 89 cGy). HTO exposure suppressed acetone precipitable fractions of HMG 1 and 14 by about 40% each and HMG 17 to a greater extent, whereas acetone-HCl precipitable HMG protein fractions were not affected. This report discusses consequences of changes in HMG proteins induced by exposure to tritium, thereby, reflecting an expression of radiotoxicity of tritium β-particles at molecular level in a biological system.

Key Words: Tritiated water (HTO), Chronic low-dose exposure, Liver HMG proteins, Swiss albino mice

Introduction

The study of low-level tritium toxicity is relevant to human safety. It is estimated that by the end of this century the man-made environmental tritium inventory, due to large scale induction of various nuclear reactor technologies, is likely to become equal to or exceed that from natural sources. Thus, human exposure to environmental tritium is expected to be at least at the level of background radiation (Charsten 1979, 1982, Feinendegen 1967, Wimber 1966). Exposure to tritium has been shown to introduce biochemical changes (Commerford et al. 1977, Garg et al. 1981, Sharan & Srivastava 1980, 1984, Srivastava et al. 1982), induce mutations (Carsten & Commerford 1976, Carsten et al. 1977, Ikushima 1984) and cause changes in chromatin (Brooks et al. 1976, Commerford et al. 1977, 1982, Macieira-Coelho 1994). We have previously shown that chronic exposure of mice to tritiated water (HTO) caused structural alterations of hexokinase and its isozymes inhibiting their activities (Sharan & Srivastava 1980, 1984), induced strand breaks of DNA and reduced colony forming ability of bone marrow cells (Srivastava et al. 1982).

The high mobility group (HMG) proteins are the most abundant and ubiquitous non-histone chromosomal proteins of eukaryotes influencing structure and functions of

[1]Department of Microbiology, University of Munster, Münster, Germany.

[2]Institute of Medicine, Research Centre, Julich GmbH, 52425 Julich, Germany.

chromatin (Goodwin & Mathew 1982). A subclass of HMG proteins, comprising HMG 1 and 2, is associated with DNA replication while another subclass, consisting of HMG 14 and 17, is involved in transcriptionally active genes (Dorbic & Witting 1987, Goodwin & Bustin 1988).

Because tritium affects chromatin and because of the close association of HMG proteins with chromatin, we have looked into the effects of a low-dose chronic exposure to tritium on HMG proteins of liver of Swiss albino mice. The experiment was designed to mimic a situation of long duration low-dose chronic exposure of human population from tritium contaminated drinking water.

Materials and Methods

Exposure of animals to tritiated water (HTO): Low-dose, chronic exposure protocol was used in this investigation. Swiss albino mice were obtained from Assam Veterinary, Guwahati. Separately, two groups of young adult male and female mice (22 ± 1 g average body weight) were exposed to HTO (BARC, Bombay) in drinking water at a dose of 37 kBq ml^{-1} (1 μCi ml^{-1}) *ad libitum*. After a 3-week priming period, 2 males and 3 females were put together in a breeding cage which continued to get HTO drinking water *ad libitum*. Standard mouse pellet was provided as food. After weaning, the F_1 offsprings were separated but were also maintained on HTO at the same dose level. 6 to 8-week old chronically exposed F_1 animals were used in this investigation. Controls were sham-exposed, age-matched animals.

Preparation of HMG Proteins: Immediately after cervical dislocation, HMG proteins were prepared from livers of two mice by extraction with 5% perchloric acid (PCA) as described by Nicolas and Goodwin (1982) with minor modification. Briefly, HMG proteins were extracted thrice by homogenization with 5% PCA (0.6 g tissue in 1 ml of PCA) in an ice bath. The supernatants of three centrifugations (4000 × *g*, 30 min, 4°C) were pooled and mixed with 3.5 × volume of acetone in the presence of 0.3 *N* HCl to precipitate H1 and some nucleotides (acetone-HCl fraction). After collecting the above fraction by centrifugation, HMG proteins were precipitated from the supernatant by further addition of 2.5 × volume of acetone (acetone fraction). The HMG proteins were collected by centrifugation (40,000 × *g*, 60 min, 4°C). The fractions were washed with acetone, lyophilized and dissolved in 0.1 ml of 100 m*M* tris-HCl buffer (*p*H 7.4). The protein contents were assayed by the method of Bradford (1976) using BSA as a standard. Six independent experiments (replicates), each with two F_1 mice, were carried out in this investigation.

Analysis by SDS-PAGE electrophoresis and densitometric quantification: A continuous gradient (6 to 15%) SDS-polyacrylamide gel with 3% stacking gel was prepared following the method of Laemmli (1970). Fifty μg protein samples were loaded onto the gels and the electrophoresis was carried out at 27 mA constant current for 60 min followed by 55 mA constant current for 90 min. After a 10 min fixation in 20% trichloroacetic acid, the gel were stained overnight with 0.125% coomassie brilliant blue and then destained. Various HMG protein bands were quantified as per cent of total peak area (optical density × full width at half maximum (FWHM); OD × mm) with the help of a Bio-Rad transmission/reflection densitometer using the gel electropherogrammes obtained from six independent PAGE runs of isolated HMG fractions.

Results and Discussion

In this investigation, the endeavour has been to mimic a situation of humans being exposed to long-duration low-dose chronic tritium by way of HTO contaminated

drinking water. Such situation may occur in parts of industrialized nations or in the vicinity of nuclear installations. To achieve this, in this investigation, young adult parents were chronically exposed for three weeks, allowed to mate under similar exposure conditions, and raise next generation which also continued to be exposed to low-dose chronic tritium β-radiation. In this situation, the F_1 generation had two components of tritium β-irradiation—one due to organically bound tritium carried through F_0 and the other due to the tritium β-exposure for 6-8 weeks in F_1.

The high mobility group (HMG) proteins were chosen as the parameter to assess the molecular consequences of such β-irradiation because, (i) these are regulatory proteins influencing the structure and function of chromatin (Dorbic & Wittig 1987, Goodwin & Bustin 1988), and (ii) the effects of tritium β-irradiation on other components of chromatin (DNA and histones) have already been studied in details (Commerford et al. 1977, Commerford et al. 1982). To the best of our knowledge, effects of tritium β-irradiation on HMG proteins have not been investigated into.

The dose of β-irradiation to F_1 mice due to long-duration low-dose chronic HTO ingestion was calculated as detailed in our earlier publication (Sharan & Srivastava 1984). Briefly, taking into account the 75% water content of the liver the actual dose rate to the liver came out to be 2.73 cGy day^{-1} (0.55 cGy day^{-1} due to the organically bound tritium and 2.18 cGy day^{-1} due to HTO ingestion in F_1) in the F_1 mice (Dobson & Kwan 1976). With 6.0 ± 0.2 ml drinking water as average daily intake (Sharan & Srivastava 1984) and 1.1 days as the biological half life of HTO for a mouse, HTO equilibrated in the mice in approximately 7 days after which a constant and chronic β-irradiation field existed. Therefore, the liver of mice of F_1 generation, used in these experiments, had accumulated a tritium β-dose of 89 cGy

Figure 1 shows the results of SDS-PAGE analysis. The cellular proteins remaining after 5% PCA extraction is shown in lanes I. Lanes II represent the proteins which are soluble in 5% PCA and were precipitated by acetone-HCl while lanes III show the proteins precipitated after further addition of acetone to the extract (C: unexposed control and HTO: exposed to tritiated water). In the absence of commercially available HMG proteins for use as standards in SDS-PAGE, HMG proteins purified by Dr G H Goodwin were used as standards (figure 1 – Std. HMG). The extraction was apparently complete as lanes I show almost no proteins of lanes II and III. Parts of HMG 1 and 14 and most of HMG 2 were precipitated in the acetone-HCl fraction (Lanes II), a step earlier than expected according to the method described by Nicolas and Goodwin (1982). Remaining HMG 1 and 14, and a faintly visible band of HMG 17 (more clearly detectable by densitometric scan; see figure 2 below) were found in the acetone fraction (Lanes III). Two other protein bands between HMG 2 and HMG 14 and one prominent band adjoining HMS 14 are likely histones (Lanes II & III); HMG and histone proteins, being basic in nature and associated with chromatin, tend to coprecipitate.

The densitometric quantification of the HMG protein bands on the gels are shown in figure 2 as mean ± SD of six independent experiments. Figure 2A depicts the quantities of various HMG proteins in the acetone-HCl fraction (figure 1-II) and figure 2B shows those in the acetone fraction (figure 1-III). In the former, the HMG proteins from unexposed as well as HTO exposed mice livers showed no quantitative differences (figure 2A). In the latter, amounts of HMG 1, 14 and 17 were significantly reduced ($P \leq 0.0001$; Student's t-test) in the HTO exposed mice liver as compared

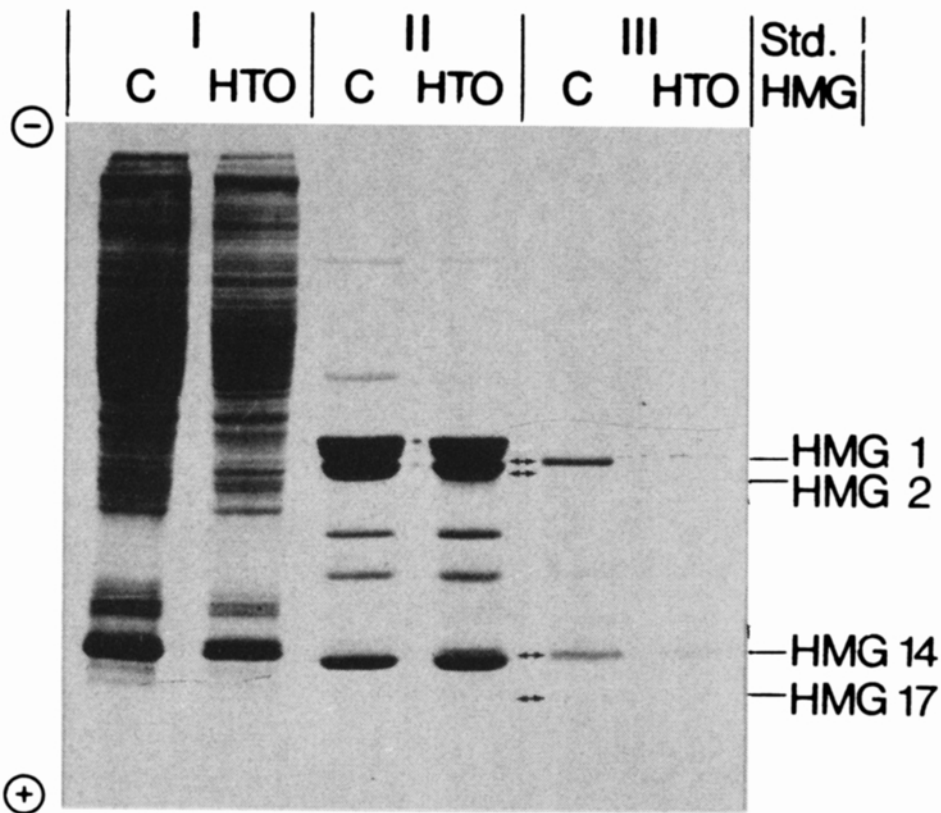

Figure 1 A 6 to 15% continuous gradient SDS-polyacrylamide gel showing protein bands stained with coomassie brilliant blue. Lanes I show 5% PCA-non-extracted proteins of liver of mice; lanes II show proteins extracted by 5% PCA and precipitated by 3.5 × volume acetône in the presence of 0.3*N* HCl (acetone-HCl fraction); and lanes III show proteins precipitated by further addition of 2.5 × volume acetone (acetone fraction). Lanes marked C and HTO are unexposed controls and tritiated water exposed groups, respectively. The positions of standard HMG proteins (1, 2, 14 and 17) are shown in Std. HMG lane.

to the unexposed controls (figure 2B). The co-precipitated histone bands have been ignored in the quantification of HMG proteins.

Precipitation of parts of HMG 1 and 14 and most of HMG 2 of liver of Swiss albino mice in the acetone-HCl fraction (figure 1-II) is unlike that reported for other cell lines and tissues (Goodwin & Bustin 1988). The remaining HMG 1, 14 and 17 were, however, precipitated in the acetone fraction in accordance with Nicolas and Goodwin (1982). The globular nature of HMG 1 and 2 which may get denatured during extraction with 5% PCA (Goodwin et al. 1978, Nicolas & Goodwin 1982), could possibly be one of the reasons of their precipitations by acetone-HCl. Alternatively, there might exist two subtypes of HMG 1 and 14 in liver of mice with differential radiosensitivities. Possible existence of two subtypes of HMG proteins has been suggested (Nicols & Goodwin 1982). The subtype precipitated in the acetone-HCl fraction was resistant to tritium β-irradiation (figures 1-II & 2A) while the subtype precipitated in the acetone fraction was sensitive (figures 1-III & 2B).

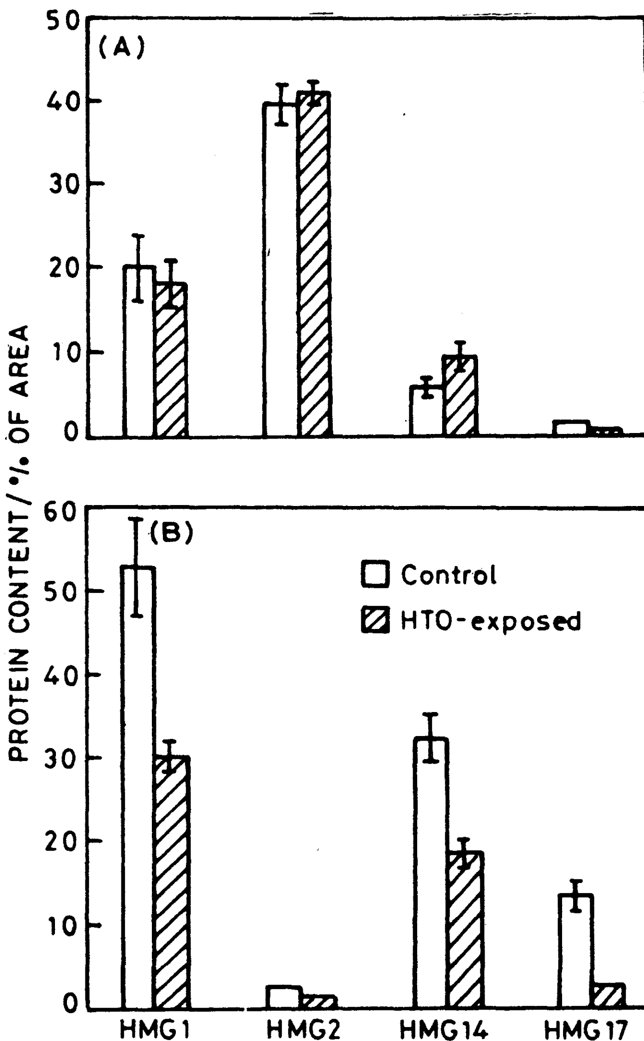

Figure 2 Densitometric quantification (mean ± SD; n = 6) of HMG proteins: A—acetone-HCl fraction (figure 1-II) and B—acetone fraction (figure 1-III). The quantification has been done as per cent of total peak area (OD × mm). The quantities of HMG proteins in the control and HTO exposed groups are statistically the same in panel A while in panel B the HMG proteins from HTO exposed group are significantly ($P \leq 0.0001$) reduced as compared to the controls.

The reduction in quantities of tritium sensitive subtypes of HMG 1 and 14 was statistically significant ($P \leq 0.0001$) in HTO exposed mice. HTO exposure resulted in reduction of about 40% of HMG 1 (from 52.67+6 to 19.8+2.67% OD × mm) and HMG 14 (from 31.76±3 to 18.33±1.87% OD × mm) (figures 1-III & 2B). The reduction in the amount of HMG 17 in the HTO exposed mice was measured to be even more pronounced (about 80% of the control: from 12.67±1.33 to 2±0% OD × mm:figures 1-III & 2B). Because of very low cellular concentration of HMG 17 which gives a faint band on the gel, the quantification of HMG 17 may be a bit exaggerated.

Since only a part of HMG 1 was tritium sensitive while the other part of it as well as HMG 2 were tritium resistant, it appears that tritium β-radiotoxicity is only partially manifested through this subclass of HMG proteins. On the contrary, the subclass comprising HMG 14 and 17, believed to be associated with maintenance of gene activity (Dorbic & Witting 1987, Goodwin et al. 1978), respond sensitively to tritium. Loss of HMG 14 and 17 made chromatin resistant to DNase I cleavage indicating alterations in the structure of chromatin due to this depletion (Schneeweiss et al. 1994, unpublished results). Our present results, therefore, suggest that tritium radiotoxicity is primarily manifested through HMG 14 and 17 by altering chromatin structure which obviously affects the gene activity (Dorbic & Wittig 1987, Goodwin et al. 1978, Goodwin & Bustin 1988). We hypothesize that HMG proteins may be an important factor in manifestation of molecular consequences of tritium radiotoxicity in biological systems. How these changes influence the reported high rate of tritium induced genetic mutations is being investigated.

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