TRITIUM β-RADIATION INDUCTION OF CHROMOSOMAL DAMAGE: A CALIBRATION CURVE FOR LOW DOSE, LOW DOSE RATE EXPOSURES OF HUMAN CELLS TO TRITIATED WATER

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ABSTRACT

Radiation exposures from tritium contribute to the occupational radiation exposures associated with CANDU reactors. Tritiated water is of particular interest since it is readily taken up by human cells and its elimination from the body, and, consequently, the radiation exposure of the cells, is spread over a period of days. Occupational exposures to tritiated water result in what are effectively chronic β-radiation exposures. The doses and dose rates ordinarily used in the definition of cellular responses to radiation *in vitro*, for use in biological dosimetry (the assessment of radiation exposures based on the observed levels of changes in the cells of exposed individuals), are usually much higher than for most occupational exposures and involve radiations other than tritium β-rays. As a result, their use in assessing the effects from tritiated water exposures may not be appropriate. We describe here an *in vitro* calibration curve for chronic tritium β-radiation induction of reciprocal chromosomal translocations in human peripheral blood lymphocytes (PBLs) for use in biodosimetry.

INTRODUCTION

Tritium exposures contribute to the occupational radiation exposures that are associated with CANDU reactors and much of the concern that is expressed by the public in discussions of the safety of nuclear power stations in Canada. Consequently, a clear understanding of the effects of tritium β-rays on human cellular material at the dose and dose rate levels that might be experienced by atomic radiation workers in such facilities is important. This is particularly so, given the wide range of estimates of the relative effectiveness of tritium β-rays that have been reported in the scientific literature [1].

The most significant exposures in the occupational context are to tritiated water (HTO). Since HTO is taken up readily by cells, and is eliminated over an extended period, the

exposures are chronic in nature: doses are accumulated at relatively low dose rate over several days. Reference curves derived from cells exposed *in vitro* are the norm in the evaluation of radiation exposures using biological dosimetry, that is, assessing the exposure based on the level of an induced effect in the cells of the exposed individuals. For most cellular effects used in such work, the dose response relationship is best described by a linear-quadratic relationship ($y = \alpha D + \beta D^2$, where y is the yield of events, and D is dose). The exposures used in defining these calibration curves are usually acute (delivered over a short period of time at high dose rate). One of the limitations of such curves is that they do not lend themselves particularly well to the definition of the linear portion of the induction curve at low doses or at low dose rates. Consequently, they are inappropriate for evaluating HTO exposures.

The work described in this report was undertaken to define a calibration curve specifically for HTO exposures in a dose range and at a dose rate that would allow us to stay within the linear portion of the dose response curve. This, in turn, allows for better definition of the α–coefficient of the induction curve.

The end-point used in this study is reciprocal chromosomal translocation. These cellular anomalies are rearrangements of cellular DNA, brought about by the incorrect rejoining of broken chromosomes, themselves a consequence of radiation damage. Reciprocal translocations are characterized by cellular stability (they do not necessarily result in cell death), making them the end-point of choice for evaluation of acute exposures long after their occurrence, or for multiple exposures over an extended period. This does not, however, preclude their use in the evaluation of acute exposures.

While the doses and dose rates used here are still well above occupational levels, they were chosen to be low enough to keep the induced levels of effect within the linear portion of the induction curve, a necessity in defining the induction rate at low doses.

METHODS

Blood from a healthy male donor was collected by venipuncture in heparinized vacutainer tubes and maintained at room temperature until treatment. To mimic the conditions to which cells *in vivo* would be exposed, aliquots of blood were diluted 1:1 with culture medium (RPMI 1640, containing 20% (v/v) fetal bovine serum, 2mM glutamine , 100 I.U./mL penicillin, 100 µg/mL streptomycin), prewarmed to 37°C. The culture medium was spiked with tritiated water at activity concentrations calculated to deliver 0.3, 0.6 or 0.9 Gy over a 48 hour incubation period. A modification of the methods of Scarpa et al. [2] and Prosser et al. [3], assuming lymphocytes to be 82% water and correcting for the non-aqueous components of the medium, was used in dose calculation. The suspensions were incubated at 37°C in sealed tubes with continuous, gentle agitation.

The isolation of the lymphocytes from the whole blood mixture using standard density-based separation methods [4] was begun at a fixed time before the end of the exposure.

The tritiated water exposure was ended effectively when the separated cells were removed from the gradient and diluted in a large volume (45 mL) of non-tritiated medium. The cells were washed three times, sufficient to reduce the residual tritium in the supernatant fluid to background levels.

The washed cells were resuspended in fresh medium containing phytohemagglutinin (PHA) to stimulate cell growth, and incubated at 37°C in a humidified atmosphere containing 5% CO_2 in air. The procedures for preparing metaphase spreads from the cultured cells were as described in Lin et al. [5]. Reciprocal chromosomal translocations involving chromosomes 1, 2 and 4 were scored using fluorescence *in situ* hybridization, a technique that allows ready microscopic identification of rearranged chromosomes [6,7]. The chromosome-specific fluorescent tags used were from Vysis Inc. (Downers Grove, IL 90515).

For this work the scoring criteria were those employed by Lucas et al. [7]. Only the results for complete reciprocal translocations are reported here. Because only events involving chromosome 1, 2 or 4 were measured, a correction factor to scale to full genome equivalents is applied [6]. Curve fitting of the data was carried out using SigmaPlot (Jandel Scientific Software, San Rafael, CA 94901).

RESULTS AND DISCUSSION

The numbers of metaphases scored and reciprocal translocation events observed following chronic exposures to tritiated water are shown in Table 1. A total of 21 117 metaphases were scored in tritiated water exposed cells. The number shown for the control (zero dose) is a composite number for several donors, and includes 4 318 from the specific donor whose cells were used in the HTO work.

The frequencies of reciprocal events, corrected to full genome levels, are plotted in Figure 1. The best fit of the data to a simple linear regression model ($y = C + \alpha D$) is indicated by the plotted line. The α-coefficient is estimated at 0.026 +/- 0.002 translocations per cell per Gy. The estimate for the y-intercept, **C**, is 0.003 +/- 0.001 translocations per cell.

Estimates for the α-coefficient, as defined by the initial slope of the induction curve, for radiations of other qualities have been made. The values obtained, 0.023 ± 0.005, 0.031 ± 0.001, and 0.035 ± 0.005 translocation per cell per Gy for ^{60}Co γ-rays [7], ^{137}Cs γ-rays [8], 250-kVp X-rays (Lucas, unpublished data), respectively, were obtained from lymphocytes exposed under somewhat different conditions, and, consequently, are not strictly comparable. The data for ^{137}Cs γ-rays [8] are plotted in Figure 1. Definition of an estimate for the relative biological effectiveness (RBE) for tritium β-rays for this sensitive, and very relevant end-point with respect to radiation exposures, must await an analysis of data from cells exposed to a reference radiation under the conditions described in this work. These were designed to approximate the conditions that the lymphocytes of an exposed individual would experience *in vivo*. The cells were supplied with nutrients

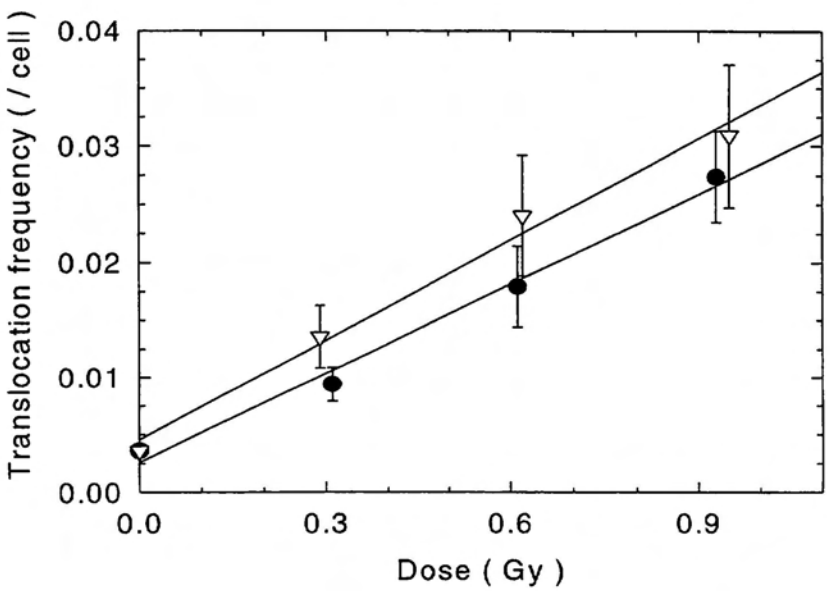
in a medium designed to maintain cellular metabolic capacity during the prolonged exposure. The medium was buffered to keep the pH in the physiological range and the blood/medium

Table 1
Reciprocal translocation induction in human lymphocytes by exposure to HTO at 37°C.

Dose (Gy)	Metaphases scored	Full genome equivalents*	Number of translocations observed	Translocation frequency per cell (1 std.dev.)
0.00	25 435	8 902	37	0.0042 (0.0007)
0.30	12 502	4 376	41	0.0094 (0.0015)
0.61	4 156	1 455	26	0.0179 (0.0035)
0.93	5 319	1 862	51	0.0274 (0.0039)

* Because chromosomes 1, 2 and 4 together represent only a fraction (0.35) of the total chromosome length in the cell, this correction factor is applied to allow the frequency to be expressed in terms of whole cells.

Figure 1
Induction of reciprocal translocations in human lymphocytes by tritium β-rays (•) and ^{137}Cs γ-rays (∇) under different exposure conditions (see text).



suspensions were held at normal body temperature, 37°C. This was not the case in the earlier work.

The dosimetry of tritium β-ray exposures to HTO is based on three critical assumptions; the HTO diffuses freely and rapidly into the cells, the water content of the cells is known and the distribution within cellular compartments is uniform. There is no disagreement in the literature that, for the lengths of exposures that are ordinarily used, the diffusion of HTO into cells is sufficiently rapid to be assumed to be instantaneous. In the absence of specific information on the distribution of intracellular HTO, its uniformity remains an unsubstantiated assumption. The water content of cells is another matter. Values for mammalian cells in general range from 45% (9) to 84% (10). The value used in this work, 82% (2), is the only specific estimate for human lymphocytes known to us. Clearly, any uncertainty in this value will reflect on the results obtained. Methods for measuring the water content of cells under the conditions used for our *in vitro* exposures are being assessed. When completed these measurements will allow us to define with confidence the α-coefficient for low dose HTO exposures. This together with the definition of the *in vitro* induction curve for the reference radiation under the same normal physiological conditions will allow us to provide a sound estimate of the RBE of tritium β-rays for this very relevant and sensitive biological end-point.

REFERENCES

1. Straume, T.; Carsten, A.L., "Tritium radiobiology and relative biological effectiveness", Health Phys. 65: 657-672, 1993.

2. Scarpa, G.; Vulpis, N.; de Santis, M.E.; Vulpis, G., "The dose absorbed by lymphocytes irradiated *in vitro* with tritiated water", Phys. Med. Biol. 26: 1137-1144, 1981.

3. Prosser, J.S.; Lloyd, D.C.; Edwards, A.A.; Stather, J.W., "The induction of chromosome aberrations in human lymphocytes by exposure to tritiated water *in vitro*", Radiat. Prot. Dosim. 4: 21-26, 1983.

4. Boyum, A., "Separation of leukocytes from blood and bone marrow", Scand. J. Clin. Lab. Invest. 21: 77, 1968.

5. Lin, C.C.; Draper, P.N.; DeBraekeleer, M., "High resolution chromosomal localization of the B gene of the human β–globin gene complex by *in situ* hybridization", Cytogenet Cell Genet. 39: 269-274, 1985.

6. Lucas, J.N.; Awa, A.; Straume, T.; Poggensee, M.; Kodoma, Y.; Nakano, M.; Ohtaki, K.; Weier, U.; Pinkel, D.; Gray, J.; Littelefield, G., "Rapid translocation frequency

analysis in humans decades after exposure to ionizing radiation", Int. J. Radiat. Biol. 62: 53-63, 1992.

7. Lucas, J.N.; Hill, F.; Burk, C.; Fester, T.; Straume, T., "Dose-response curve for chromosome translocations measured in human lymphocytes exposed to ^{60}Co gamma-rays", Health Phys. 68: 761-765, 1995.

8. Lucas, J.N.; Hill, F.S.; Burk, C.E.; Lewis, A.D.; Lucas, A.K.; Chen, A.M.; Sales, F.C.; Straume, T., "Dose-response curve for chromosome translocations induced by low-dose rate ^{137}Cs gamma-rays", Health Phys., submitted for publication.

9. Balanov, M.I.; Zhesko, T.V., "The water content in tissues and cells in mammals", Sechenov Physiological Journal of the USSR 75:963-969, 1989.

10. Yamaguchi, T.; Muraiso, C.; Furono-Fukushi, I.; Tsuboi, A., "Water content in cultured mammalian cells for dosimetry of beta-rays from tritiated water", J. Radiat. Res. 31: 333-339, 1990.