RBE OF TRITIUM MEASURED IN HUMAN CELLS USING CYTOGENETIC ENDPOINTS

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ABSTRACT

Radiations differ in the way their energy is deposited in biological material. Because of this, a weighting factor, based in part on the relative biological effectiveness (RBE), is used to normalize doses of different radiation qualities. An evaluation of the RBE for tritium β-rays, relative to other radiation qualities, is clearly important to the nuclear industry since tritium exposures contribute to total occupational doses associated with CANDU reactors. However, there is a significant range of published RBE values for tritium relative to both X-rays and γ-rays in the literature (1). In an effort to sort out some of the ambiguity associated with the effects of tritium, we completed a series of experiments using cultured human diploid fibroblast strains from four different donors. A measurement of the micronucleus induction frequency for both acute and chronic dose delivery resulted in a composite RBE value of approximately one for all strains evaluated. An RBE value of one has also been measured using the induction of this same endpoint in isolated human peripheral blood lymphocytes exposed to 65 kVp X-rays and tritium β-rays.

INTRODUCTION

Micronuclei (MN) are formed within a cell as a result of damage to the cell's genetic material, the DNA, that has gone unrepaired. Micronuclei contain either small fragments of DNA, or entire chromosomes, that are segregated separately from the main nucleus during cell division. As the level of damage increases with exposure dose, so too does the amount of unrepaired damage and, as a consequence, the measured MN frequency. Since these small nuclei are formed as a result of residual damage, their induced frequency can be used to measure the effect of many damaging agents including radiation of different qualities. Standard methods (2) were used to measure MN present in cells during the first cell division following repair. That is, the MN were scored in cells with two nuclei, a marker of cells that have undergone only one division following DNA insult. MN in these cells reflect the damage that has gone unrepaired.

Studies have shown that individuals differ in their sensitivity to the effects of radiation, as measured *in vitro* using cultured cell lines derived from them (3). These differences may be due, in part, to the inherent genetic variation in the competence of the cellular DNA repair systems observed in cell lines established from different donors. In order to test this variability we measured the MN induction levels in cultured human dermal fibroblast cell strains from four different donors, obtained from the Human Genetic Mutant Cell Repository, Coriell Institute for Medical Research, Camden, New Jersey. Two of the cell strains assayed were from "radionormal" individuals (people with no identified sensitivity to radiation), while the other two cell lines were established from individuals homozygous for the genetic condition ataxia telangiectasia (AT), a syndrome associated with radiosensitivity (4). All four strains were evaluated for variation in growth under experimental handling conditions to remove any potential bias in our results associated with tissue culture parameters outside the experimental considerations. A description of the cell strains is found in Table 1.

An important factor to be assessed when assigning RBE values to radiations of different qualities is the rate at which the dose has been delivered. During occupational exposure, doses are delivered in a chronic fashion as relatively small incremental amounts spread over a long period of time. In rare, accident situations the entire dose can be delivered all at once. The way in which individuals, and cells in culture, respond and ultimately repair the damage introduced under the two exposure conditions might be quite different. We tested this using

two rates of dose delivery. The exposures designed to mirror the chronic situation were delivered at a dose rate of 0.0026 Gy/min for all radiation qualities evaluated. An acute rate of 0.135 Gy/min provided a 50-fold increase in the radiation dose delivery. In all cases, the radiation doses, regardless of radiation quality, were delivered at normal body temperature (37°C) so as to mimic DNA repair systems *in vivo*. However, to compare the effect of total dose regardless of dose rate the doses for all experiments were consistent. The cells from the radionormal individuals were exposed to doses up to 2.0 Gy in the acute experiments and to 4.0 Gy in the chronic experiments. This distinction in doses was used to reflect the dose rate modifying factor which allows for the increased repair time when the dose is delivered chronically. The same approximate level of residual damage is measured at the higher chronic doses as at the lower acute doses resulting in an equivalent biological response. In the case of the cells from the radiosensitive AT individuals, the dose range was 0.25 Gy to 1.0 Gy for both exposure rates. This decrease in doses was in keeping with the reduced level of both repair capacity and repair competence in these cell lines.

RESULTS

The RBE for tritium β-rays was evaluated using 250 kVp X-rays and ^{60}Co γ-rays. The doses and dose rates for X-rays and γ-rays were established using an Ionex Dosemaster 2590 Ion Chamber. These dose rates were confirmed by the simultaneous exposure of lithium fluoride TLDs attached to the tissue culture flasks. The dose rate from the ^{3}H in the tritiated water (HTO) used was taken as $5.48 \times 10^{-2} \times C$ Sv/min, where C is the concentration of tritium in the water in gigabecquerel per millilitre (0.203 x C for C in mCi/mL). From the activity concentration, the dose rate was measured on the assumption that the added HTO was distributed uniformly throughout the culture medium, the nucleus and the cytoplasm of the cell, that the cells were 80% water (5) and that the equilibration time between cell water and HTO suspension was short compared to the exposure time. The fibroblasts were exposed to all of the different radiations as an attached confluent monolayer. To end the exposure, tritiated medium was removed and the cells were washed repeatedly with fresh medium until the activity in the wash supernatants was reduced to background levels, as determined by scintillation counting.

The MN frequency was determined by scoring, whenever possible, 1000 binucleate cells (BNC) per dose point. To facilitate the scoring, the cells were stained with a DNA intercalating dye and evaluated using fluorescence microscopy. The data for each strain and each radiation type was subjected to non-linear regression (curve-fitting) using the SigmaPlot 5.0 computer software package (Jandel Scientific). This analysis involved an iterative, least squares procedure which minimized the sum of the squares of the differences between the equation values and the data values. The equation used was log (induced MN frequency) = $c + \alpha D + \beta D^2$, where α and β are constants and c is the intercept. The induced MN frequencies were first corrected for the spontaneous frequency in each experiment. The spontaneous MN frequency measured in the normal cell strains was approximately 20 per 1000 binucleate cells. The cell strains from the AT donors were found to have spontaneous frequencies at least 5-fold higher than those in the normal cell lines. A summary of the spontaneous frequencies for all four of the cell strains is found in Table 2. Typical graphical representations of the results are included in Figures 1 and 2.

The RBE is defined as the ratio of the doses of two different radiation qualities that result in the same level of induction of the endpoint measured. In this series of experiment,s the effect on MN induction for both the tritium β-rays and ^{60}Co γ-rays was compared to that resulting from exposure to 250 kVp X-rays. In the situation where β in the non-linear regression equation is negligible, applicable at low dose levels, the RBE can be evaluated from the ratio of the α coefficients, the fitted slopes of the linear portion of the curves. The calculated composite value for the RBE comparing HTO β-rays and 250 kVp X-rays for each strain evaluated using the above method of analysis is found in Table 3. Calculated values for ^{60}Co γ-rays are summarized in Table 4.

DISCUSSION

As with most cytogenetic endpoints used to evaluate the effects of DNA-damaging agents, the induced frequency is measured against a background of spontaneous events. The most striking feature of the spontaneous levels measured in these four strains is the high frequencies measured in both of the AT cell lines tested. The increased chromosomal instability found in AT strains, as indicated by spontaneously occurring DNA damage, is consistent with a reduction in DNA repair capability. In order to compensate for a reduction in repair capacity in the AT cell strains, the exposure doses chosen were lowered to give MN induction frequencies in the same general range as the normal, control cell lines. Since the AT cells are repair-deficient, regardless of the rate of radiation dose delivery, this dose reduction was applied to both acute and chronic protocols. Conversely, the repair-proficient normal cells do show an advantage on chronic dose delivery, presumbly a consequence of repair of DNA damage during the protracted exposure. To reflect this advantage, the chronic exposures were higher than the acute doses, resulting in equivalent levels of MN induction at both dose rates.

A comparison of the data obtained shows a large difference in the overall induction levels in the normal strains for acute versus chronic exposures. Acute exposures were some two-fold more effective in inducing MN per unit dose than were chronic exposures in repair-proficient cells. Conversely, in general and for most irradiations, the AT cell lines tested did not show any dose rate reduction in the levels of induced effect. Also, a comparison of the plotted curves indicates a large difference between the normal and AT lines in their MN induction rates. At the lower doses, as indicated by the α coefficient, the AT strains have generally a 10-fold higher induction frequency than do the normal stains. This observation is consistent with the reduced capacity of AT cell lines to repair lesions that give rise to double strand breaks in DNA (6, 7).

Regardless of the differences in induction frequencies between these cell populations, the experimental results indicate that neither dose rate nor repair competence of the cells exerted any major influence on the RBE values measured using the MN assay. The composite RBE value for the effect of HTO β- rays relative to the reference radiation of 250 kVp X-rays for all four strains was determined to be 1.34 ± 0.14 for the acute exposures and 1.27 ± 0.27 in the case of the chronic dose rate.

The RBE values calculated for the effect of ^{60}Co γ-rays compared to the reference standard of 250 kVp X-rays showed much the same with all four strains giving equivalent values. However, it is interesting that the measured RBE values were significantly lower than those measured for tritium with 250 kVp X-rays as the reference radiation. The calculated composite RBE values were 0.52 ± 0.05 for the acute exposure and 0.82 ± 0.17 for the chronic dose rate when the effects of γ-rays were compared to those of X-rays. Extrapolating from these values, it would appear that using ^{60}Co γ-rays as the reference radiation against which to measure the effects of HTO would have given RBEs of 2.6 and 1.5 for acute and chronic exposures, respectively. When evaluating RBE data for tritium β-rays, it is imperative to identify clearly the reference radiation.

In a subsequent series of experiments, to establish validation standards for our tritium exposure methodologies and dosimetry calculations, we once again measured the effects of HTO on the level of MN induction. For these experiments the cells exposed were isolated human peripheral blood lymphocytes collected from a volunteer donor at CRL. In order to approximate microdosimetrically the damage deposition of the tritium β-rays, following discussions with health physics experts within the Radiation Biology and Health Physics Branch at CRL, a reference radiation source of 65 kVp X-rays was used. Due to equipment constraints, to maintain the exposure temperature to close to 37°C, all radiation doses were delivered acutely. The dose rate used throughout was approximately 100 cGy/hour, with the tritium β-ray dose calculated as previously described. Once again, the residual HTO concentration in the wash supernatants was monitored using standard techniques in scintillation counting. The measured induced MN frequencies are summarized in Table 5. The RBE values calculated from these data is unity, in keeping with the results previously obtained using cultured dermal fibroblast cell strains.

Finally, we have initiated a collaborative effort with members of the Cytogenetics Group at the Lawrence Livermore National Laboratory to better define the risks associated with tritium exposure in the low dose range. This collaboration has used the measurement of chromosomal translocations as the identifiable endpoint. Translocations are aberrations that occur when different chromosomes within a cell exchange segments of DNA resulting in a measurable change in the DNA sequence along each affected chromosome. Translocations differ from micronuclei in that they are changes in cellular DNA that may persist in the cells of exposed individual long after the inducing radiation exposure (8). In this way, they are believed by some to provide an integrating lifetime measurement of the total cumulative dose received by a particular individual. This effort serves two main purposes and complements the work already completed by both laboratories. It is designed to provide a calibration curve for tritium β-rays for use in biological dosimetry of individuals chronically exposed to tritiated water and to provide an estimate of the RBE of tritium at doses and dose rates that are low enough to be relevant to occupational exposures, and for an end-point that is relevant to long-term health risks.

Preliminary results indicate that the experimental conditions under which the cells are exposed to radiation may be critical in defining an appropriate induction curve for biological dosimetry purposes. Cells held and exposed at room temperature (the standard conditions used in the past by our collaborators at LLNL) appear to show higher translocation frequencies than cells, in the same experiment, held and exposed at the physiologically normal temperature of 37°C. These results are summarized in Table 6.

CONCLUSION

The identification of a radiation weighting factor for tritium is of importance to the Canadian nuclear industry. Valid estimates of the measured effectiveness of tritium as an inducing agent for cellular end-points associated with adverse biological effects is important, not only to radiation protection practices, but also to the financial considerations that follow from these in the operation of a nuclear power station. We have shown that in the definition of an RBE value, close attention must be paid to the physical conditions under which the experiment is run and to the reference radiation against which the effect of the tritium is being measured. The experimental results described here do much to validate the methodologies used in our tritium exposures. Our results, which do support an RBE of one for the effect of tritium compared to X-rays, are in keeping with recent ICRP recommendations related to tritium exposures.

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Cell Line	Genotype / Phenotype	Description of donor
GM0038	Presumptive normal	9-year old female
GM2185	Presumptive normal	36-year old male
GM2052	AT homozygous	15-year old female
GM2531	AT homozygous	5-year old male

Table 1 Description of the human fibroblast cell lines used in this work.

Cell Line	Phenotype	Spontaneous Frequency of MN per 1000 BNC (± S.E.)
GM0038	Normal	11.4 ± 1.8
GM2185	Normal	27.1 ± 5.9
GM2052	Radiosensitive	179 ± 41
GM2531	Radiosensitive	107 ± 17

Table 2 Background frequencies of MN for each of the four cell lines used in this work.

Cell Line	Mean RBE Values	
	Acute Exposure	Chronic Exposure
GM0038	1.71	0.89
GM2185	1.14	2.02
GM2052	1.13	0.88
GM2531	1.39	1.30
Overall Mean	1.34 ± 0.12	1.27 ± 0.23

Table 3 RBE values for ^{3}H β-rays relative to 250 kVp X-rays for each of the four lines used under each of the exposure conditions.

Cell Line	Mean RBE Values	
	Acute Exposure	Chronic Exposure
GM0038	0.41	0.48
GM2185	0.57	1.12
GM2052	0.44	0.55
GM2531	0.65	1.11
Overall Mean	0.52 ± 0.05	0.82 ± 0.17

Table 4 RBE values for ^{60}Co γ-rays relative to 250 kVp X-rays for each of the four lines used under each of the exposure conditions.

Dose (cGy)	^{3}H β-rays	65 kVp X-Rays
0.00	0.065	0.068
50.0	0.20	0.22
100.0	0.36	0.35
200.0	0.52	0.52

Table 5 Induction of MN by ^{3}H β-rays and 65 kVp X-rays in human peripheral blood lymphocytes

Dose (Gy)	Metaphase Cells (Full Genome Equivalents)	Translocations	Frequency (± 1 S.D.)	^{137}Cs Exposure* Frequency (± 1 S.D.)
0.0	1435 (502)	3	0.0060 (0.0035)	0.0039 (0.0008)
0.3	605 (211)	2	0.0095 (0.0067)	0.014 (0.0027)
0.6	606 (212)	4	0.019 (0.0095)	0.028 (0.0056)
0.9	812 (284)	9	0.032 (0.011)	0.041 (0.0051)

*J.N. Lucas et al., *Dose-Response Curve For Chromosome Translocations Induced By Low Dose Rate ^{137}Cs γ-rays*, Health Physics, submitted for publication.

Table 6 Preliminary data on the induction by of reciprocal chromosome translocations in human peripheral blood lymphocytes

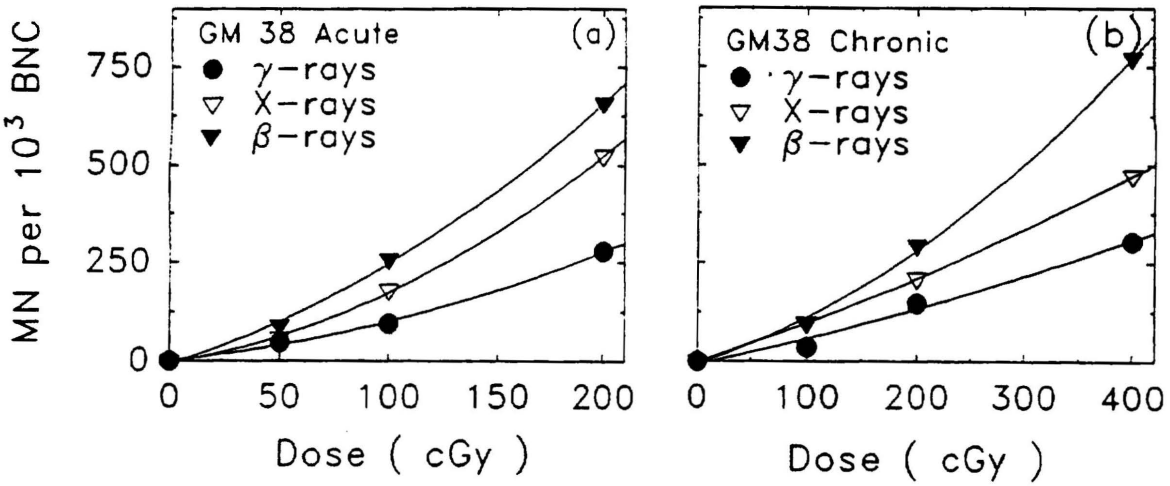

Figure 1 Dose response curves for ^{3}H β-ray-, ^{60}Co γ-ray- and 250 kVp X-ray induction of MN in a human, radionormal fibroblast cell line. (a) Acute exposure. (b) Chronic exposure.

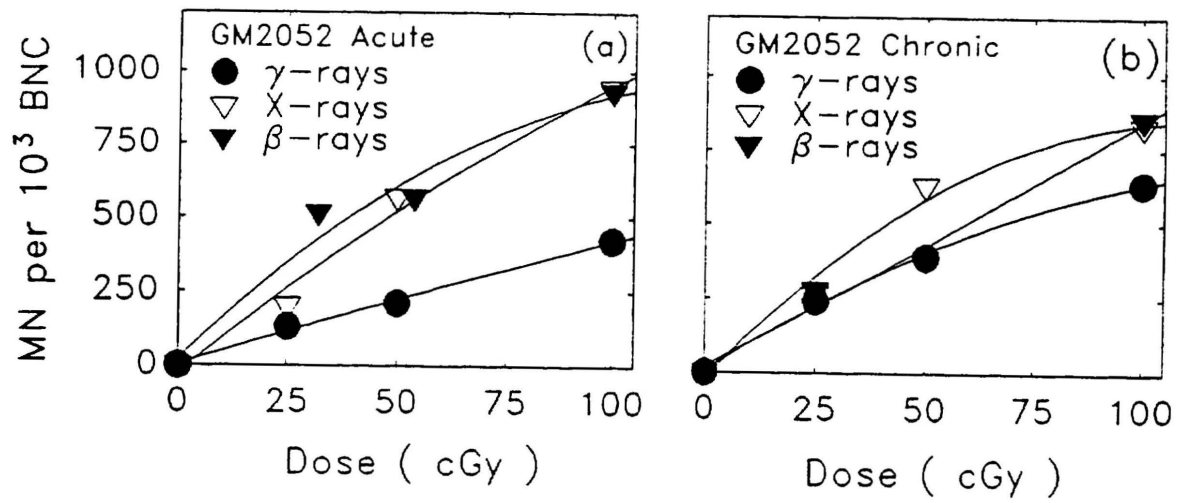

Figure 2 Dose response curves for ^{3}H β-ray-, ^{60}Co γ-ray- and 250 kVp X-ray induction of MN in a human, radiosensitive fibroblast cell line. (a) Acute exposure. (b) Chronic exposure.