

# Inverse dose-rate effect for mutation induction by $\gamma$ -rays in human lymphoblasts

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**Abstract.** In order to define further the effects of differences in recombinational proficiency on cell survival and mutation by ionizing radiation, we exposed the syngenic cell lines TK6 and WTK1 to continuous low dose-rate  $\gamma$ -irradiation. We previously demonstrated that acute X-ray exposure results in lower survival and lower mutation induction at both the thymidine kinase (*tk*) and the hypoxanthine-guanine phosphoribosyltransferase (*hprt*) loci in TK6 cells compared with WTK1 cells. These differences were attributed in part to reduced levels of recombination in the TK6 line relative to WTK1. Using a low dose rate  $^{137}\text{Cs}$  irradiator, we exposed asynchronous growing populations of these cells to  $\gamma$ -rays at 1.4, 6.7 and 2.7 cGy/h. Both cell lines exhibited a dose-rate effect on survival. Compared with acute doses, the low dose-rates also protected against mutation induction at the *hprt* locus in WTK1, but protection was inversely related to dose-rate. There was also a slight inverse dose-rate effect in TK6, with mutation induction at the lowest dose-rate exceeding that at acute exposures.

## 1. Introduction

The biological effects of low dose-rate radiation exposure are of importance to both cancer radiotherapy and radiation protection. Early studies focused on the effects of dose fractionation on cell survival, a parameter relevant to radiotherapy design. It was found that splitting an X-ray dose into two or more fractions resulted in an increase in cell survival over that seen when the same total dose was delivered as a single fraction (Elkind and Sutton 1959). This effect was observed both *in vitro* and *in vivo*, and it was hypothesized that the time between the fractions allowed the cells to repair a subset of the radiation damage, referred to as 'sub-lethal damage' (Elkind *et al.* 1967). One test of the repair hypothesis was to compare the effect of changes in dose-rate on survival of normal cells

and radiation sensitive mutants or other cell lines deficient in DNA repair. The mouse lymphoma cell line LYR, for example, showed increased survival at low dose-rate, while its X-ray-sensitive derivative, LYS, manifested only a slight dose-rate effect (Evans *et al.* 1985). Similarly, CHO K-1 cells had about a 2.5-fold increase in survival at low dose-rate compared with acute exposure, while their X-ray-sensitive mutants, *xrs-5*, *xrs-6* (Nagasawa *et al.* 1989), and *irs-20* (Stackhouse and Bedford 1993), exhibited no dose-rate effect. These sensitive cells are all believed to be deficient in double-strand break repair. Human skin fibroblasts derived from an ataxia telangiectasia (AT) patient have also been found to have no low dose-rate effect in comparison with normal human fibroblasts, which showed enhanced survival at low dose-rates (Nagasawa *et al.* 1992). AT cells are very sensitive to ionizing radiation and are thought to have a defect either in DNA repair, or in a cell cycle checkpoint that allows cells to arrest following exposure to ionizing radiation.

In contrast with the repair-deficient mutants, studies of transformed rodent cells suggest that these cells may exhibit an even greater dose-rate effect than normal cells. Transformed 10T $\frac{1}{2}$  cells, for example, cycled more rapidly than non-transformed C3H 10T $\frac{1}{2}$  cells, and showed greater sparing by fractionated  $\gamma$ -ray exposures (Zeman and Bedford 1985). Rat embryo cells transfected with the Ha-*ras* oncogene also showed a greater dose-rate effect for survival than non-transfected cells (Ong *et al.* 1993). Both these studies suggested that transformed cells have a greater capacity for repair than non-transformed cells.

The effects of dose protraction on mutation and cell transformation are perhaps more relevant to human risk estimates. These endpoints are thought to be indicators of damage and repair mechanisms that result in long-term effects such as heritable genetic change or cancer. Low dose-rate  $^{137}\text{Cs}$  irradiation of mice produced fewer hypoxanthine phosphoribosyl transferase (*hprt*) mutations in T lymphocytes than did acute

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exposures (Lorenz *et al.* 1994). Low dose-rate induction of mutation has been studied in several mammalian cell lines (reviewed in Lorenz *et al.* 1993), but a coherent picture of the effects has yet to emerge. Evans *et al.* (1985) found little effect of dose-rate on mutation at the *hprt* locus with either LY-R or LY-S cells using a dose-rate of approximately 2 cGy/h, but there was a decrease in thymidine kinase (*tk*) mutant induction with decreasing dose-rate (Evans *et al.* 1990). However, Furuno-Fukushi *et al.* (1988) reported that a dose-rate of 20 cGy/h decreased the induction of *hprt* mutants in L5178Y cells, while 6.3 mGy/h yielded an induction of *hprt* mutants intermediate between the acute and 20 cGy/h responses. A similar inversion of dose-rate effect for *hprt* mutation has been reported for V79-S cells (Crompton *et al.* 1990).

Previously we described two human lymphoblastoid cell lines, TK6 and WTK1, which were derived from the WI-L2 isolate of Levy *et al.* (1968). Following exposure to the chemical point mutagens ICR-191 and ethyl methanesulphonate (EMS) we found no differences between the two cell lines for mutation induction at either the *tk* or *hprt* loci. However, acute X-irradiation resulted in higher survival of WTK1 compared with TK6. The enhanced X-ray survival was due both to a shoulder in the survival curve of WTK1 that is not seen in TK6, and to a more shallow slope (higher  $D_0$ ) in WTK1 than in TK6. Mutation induction at the *hprt* locus was slightly higher in WTK1 than in TK6, and the dose-response was best fit by a linear-quadratic curve rather than the linear fit for TK6. At the *tk* locus, acute X-ray exposure induced 20-fold more mutants in WTK1 than in TK6. We suggested that the different responses of these cell lines may be due to different capacities for error-prone repair, perhaps involving recombination mechanisms. We have used fluorescent *in situ* hybridization analysis of mutants and an *in vitro* plasmid recombination assay to gain evidence in support of this idea (Xia, *et al.* 1994).

Several studies have reported the absence of a dose-rate effect on mutation in TK6 using tritiated water (Liber *et al.* 1985), X-ray fractionation (Grososky and Little 1985) or low dose-rate  $^{60}\text{Co}$   $\gamma$ -rays (König and Kiefer 1988). We were interested to see if the differences in radiation survival, mutation, recombination and damage repair which we had observed previously in WTK1 would be affected by low dose-rate exposures. We now report a dose-rate effect on survival in both TK6 and WTK1. In addition, an inverse dose-rate

effect on mutation induction was seen in both cell lines; that is, the highest of the low dose-rates used (14.3 cGy/h) gave the lowest levels of mutation induction, and as the dose-rate was lowered mutation induction increased. In TK6, the lowest dose-rate (2.7 cGy/h) induced more *hprt* mutants than acute irradiation.

## 2. Materials and methods

### 2.1. Cell culture

The human lymphoblast cell lines used in this study were both derived from WI-L2, which was isolated from the spleen of a boy with hereditary spherocytic anaemia (Levy *et al.* 1968). TK6 was derived from an unselected subclone of WI-L2 that was treated with ICR-1 and selected for *tk* heterozygosity (Skopek *et al.* 1978). WTK1, also a *tk* heterozygote, was selected from a different subclone of WI-L2 (Benjamin *et al.* 1991). The different responses of these cell lines to treatment with acute X-rays and chemical mutagens have been described previously (Amundson *et al.* 1993).

Cells were maintained as exponentially growing cultures in RPMI 1640 medium supplemented with 10% horse serum (heat treated for 2 h at 56°C). Penicillin (100 U/ml) and streptomycin (100  $\mu\text{g}/\text{ml}$ ) were added to the medium for some experiments. The cultures were incubated at 37°C in 5%  $\text{CO}_2$  and maintained at densities of 1–12  $\times 10^5$  cells/ml. Prior to experiments, cultures were treated for 2 days with CHAT medium (RPMI 1640 plus 10% horse serum plus  $10^{-5}$  M deoxycytidine,  $2 \times 10^{-4}$  M hypoxanthine,  $2 \times 10^{-7}$  M aminopterin, and  $1.75 \times 10^{-5}$  M thymidine) to reduce the background *hprt* mutant fraction. Unirradiated control cultures were started from the same pool of CHAT treated cells as the treated populations in each experiment immediately before irradiations were begun.

### 2.2. Irradiations

A Model Mark I-68A high dose-rate  $^{137}\text{Cs}$  source chamber irradiator (J. L. Shepherd and Associates, Inc., San Fernando, CA, USA.) was used to deliver acute exposures at a dose-rate of 99 cGy/min. Low dose-rate irradiations were performed within a humidified incubator (37°C with 5%  $\text{CO}_2$ ) using a Model 81-12 dual source beam  $^{137}\text{Cs}$  irradiator with a Model 155 attenuator

system (J. L. Shepherd) configured to produce dose-rates within the incubator of 14.3, 6.7 or 2.7 cGy/h. The dose-rate to the cells in each exposure condition was determined using a Victoreen meter and thermoluminescent dosimetry chips.

Aliquots of cells were removed from the irradiator at various time intervals and fixed in 70% ethanol for flow cytometry. Cells were stained with mithramycin and analyzed for DNA content as described previously (Crissman and Steinkamp 1982). DNA histograms were analyzed using the MultiCycle Software Package.

Immediately following the exposure period, cells were plated for survival in 96-well microtiter plates at densities from 1 to  $4 \times 10^4$  cells/well, depending on dose and cell line. A minimum of  $4 \times 10^7$  cells was removed from the main cultures in the irradiator for each point at which mutation was measured. The exposed cultures were maintained as 100–200 ml cultures in flasks under normal culture conditions (see above) for 7 days to allow full expression of *hprt*-mutant phenotype. At the start of these experiments, daily mutant fraction measurements were made of cultures irradiated with  $^{137}\text{Cs}$ , and the pattern of phenotypic expression (full expression of stable mutant fractions after 5–6 days) was not found to vary from that observed previously with other agents (Thilly *et al.* 1980, Liber and Thilly 1982, Amundson *et al.* 1993). After allowing for phenotypic expression,  $2\text{--}4 \times 10^4$  cells/well were seeded in medium with  $0.5 \mu\text{g/ml}$  6-thioguanine (6-TG) in 96-well, flat-bottomed microtiter plates. A control culture was maintained through the duration of each experiment and aliquots were seeded in the same way for the determination of background mutant fraction at the same time as each exposure point. Plates were incubated and scored for colony formation after 11 days. Mutant fractions were calculated using the method of Furth *et al.* (1981). Induced mutant fractions were obtained by subtracting the mutant fraction of the appropriate control culture from the total mutant fraction in the exposed culture. Linear fits to the data were calculated using Sigma Plot (version 5.0) and the slopes were compared using a two-tailed *t*-test.

### 3. Results

The differences in radiosensitivity between TK6 and WTK1 were reflected in the growth of the cultures during irradiation at 14.3, 6.7 and 2.7 cGy/h. During the first 72 h of exposure, the

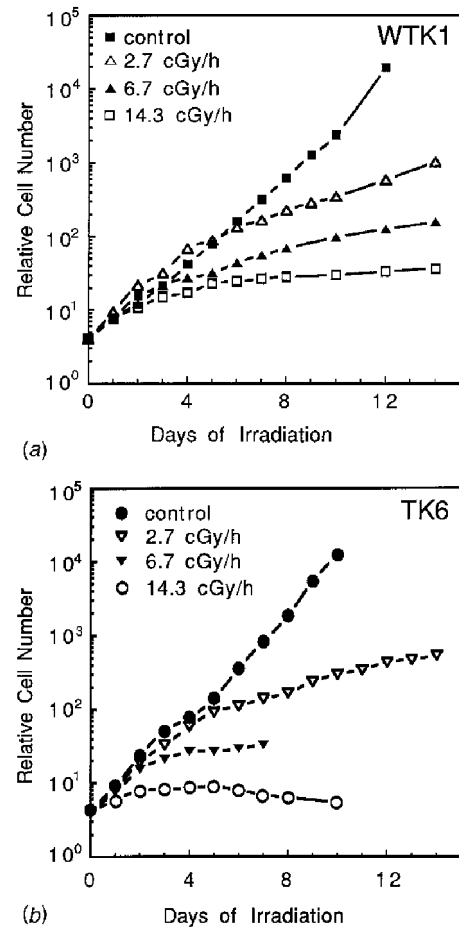
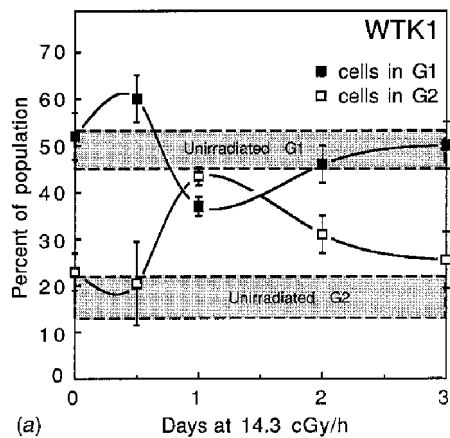


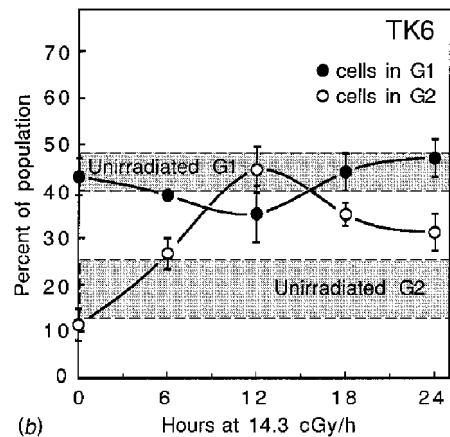
Figure 1(A). Population growth of WTK1 during irradiation in a typical experiment. Relative cell numbers were determined from daily cell counts and dilution factors. (B) Population growth of TK6 during irradiation in a typical experiment. Relative cell numbers were determined from daily cell counts and dilution factors.

population growth rate of WTK1 was not much altered at any of the dose-rates used (Figure 1(A)), while the doubling time of TK6 increased even during the first 24 h of exposure (Figure 1(B)). At the highest low dose-rate (14.3 cGy/h), TK6 populations did not increase appreciably after 2 days of exposure. However, BrdU labelled flow cytometry indicated that cells were cycling throughout the exposure periods, even at the highest doses used for the measurement of mutation. The continuation of cell division in the absence of population growth indicates an equilibrium between division and cell death or apoptosis.

Flow cytometric analysis of DNA content was performed during the course of some irradiations in order to look for trends of change in cell cycle distributions. In both cell lines cell-cycle distributions were monitored through the period of



(a)

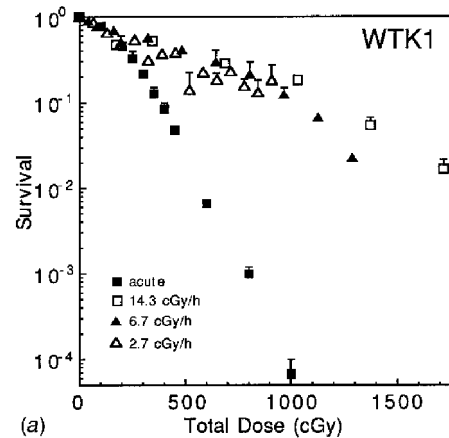


(b)

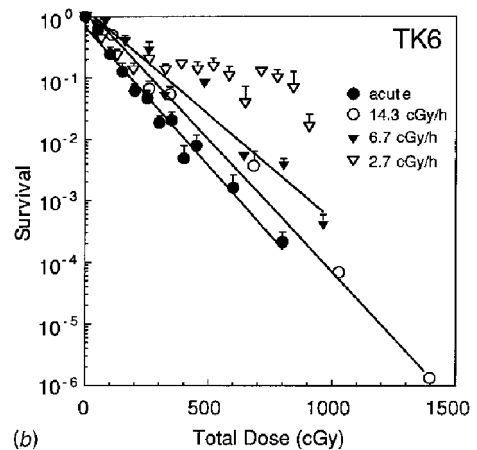
Figure 2(A). Cell-cycle distributions of WTK1 cells during irradiation at 14.3 cGy/h as determined from analysis of DNA content by flow cytometry. Each point is the average of 3–4 independent experiments and error bars are SEM. The shaded areas indicate the normal range among unirradiated control populations (mean and SEM of 19 independent determinations). (B) Cell cycle distributions of TK6 during irradiation at 14.3 cGy/h as determined from analysis of DNA content by flow cytometry. Each point is the average of 3–4 independent experiments and error bars are SEM. The shaded areas indicate the normal range among unirradiated control populations (mean and SEM of 19 independent determinations).

irradiation used for mutant fraction determination. 14.3 cGy/h was the only dose-rate at which any deviation from the distribution of unirradiated controls was observed in either cell line. In WTK1, an initial increase in cells in G<sub>1</sub> after 12 h of irradiation was followed by a loss of cells in G<sub>1</sub> and a greater accumulation in G<sub>2</sub>/M at 24 h (Figure 2(A)). The distribution then returned close to normal as irradiation continued. In TK6, a transient increase in representation of G<sub>2</sub>/M cells also occurred during irradiation at 14.3 cGy/h (Figure 2(B)).

The continuous low dose-rates used in this study



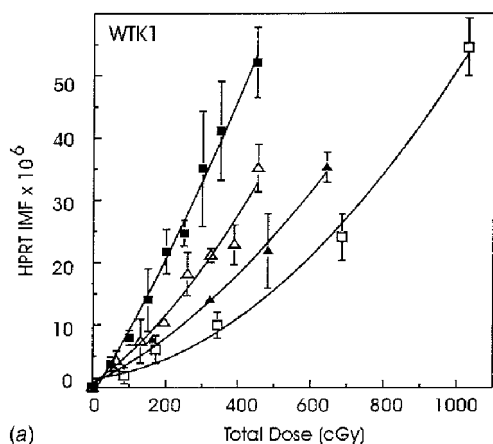
(a)



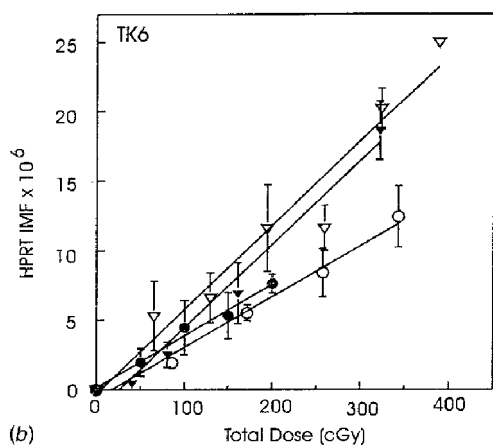
(b)

Figure 3(A). Survival of WTK1 following exposure to  $\gamma$ -rays at different dose-rates. Each point is the average of 3–9 independent experiments, and error bars are SEM. (B) Survival of TK6 following exposure to  $\gamma$ -rays at different dose-rates. Each point is the average of 3–9 independent experiments, and error bars are SEM.

all produced sparing effects on survival when compared with acute irradiation of both WTK1 (Figure 3(A)) and TK6 (Figure 3(B)). These effects were more pronounced in WTK1, the cell line showing more resistance to acute doses of radiation ( $D_0 = 90$  cGy). The survival curves for lower dose-rate exposure of WTK1 became nearly tangential to the shoulder of the acute curve, and had  $D_0$  values of 310 cGy for 14.3 cGy/h, 405 cGy for 6.7 cGy/h, and 430 cGy for 2.7 cGy/h. Despite not having a pronounced shoulder in its acute survival curve ( $D_0$  approximately 80 cGy), TK6 also showed a dose-rate effect for cell killing.  $D_0$  was 100 cGy for 14.3 cGy/h exposure, 165 cGy for 6.7 cGy/h, and 405 for 2.7 cGy/h. It should be noted that clonogenic survival assays were used to measure survival for this study. A cell may be able to undergo several doublings, thus contributing to the population growth seen in Figure 1,



(a)

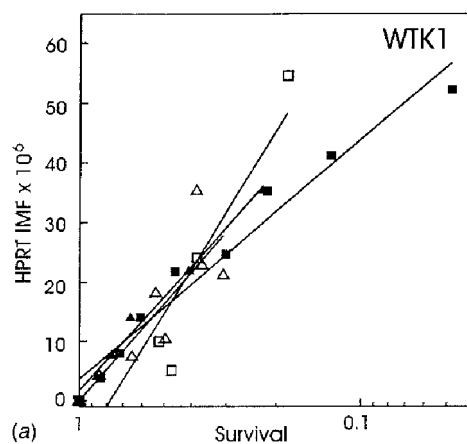


(b)

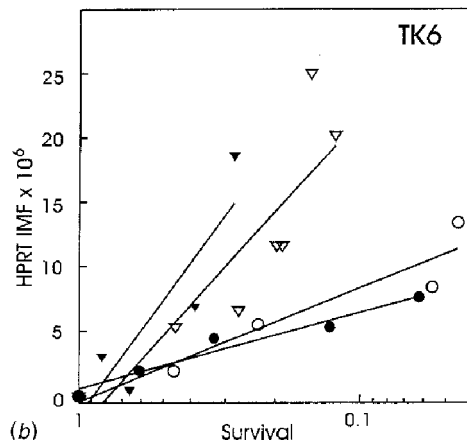
Figure 4(A). Induced mutant fraction (IMF) at the *hprt* locus in WTK1 cells by  $\gamma$ -rays at different dose-rates. Each point is the average of 3–7 independent experiments, and error bars are SEM. Background mutant fractions as determined from parallel untreated cultures ranged between  $3.3$  and  $7.8 \times 10^{-6}$  depending on the duration of the experiment and have been subtracted. Symbols are the same as those used in Figure 3(A). (B) Induced mutant fraction (IMF) at the *hprt* locus in TK6 cells by  $\gamma$ -rays at different dose-rates. Each point is the average of 3–7 independent experiments, and error bars are SEMs. Background mutant fractions as determined from parallel untreated cultures ranged between  $0.8$  and  $2.4 \times 10^{-6}$  depending on the duration of the experiment and have been subtracted. Symbols are the same as those used in Figure 3(B).

but be unable to continue dividing to form a macroscopic colony.

Mutation induction at the *hprt* locus was also reduced in WTK1 cells at all the low dose-rates when compared with acute exposures (Figure 4(A)). There was, however, an ‘inversion’ of this effect in that the lowest yield of mutants was seen with  $14.3$  cGy/h, and the highest dose response was seen with  $2.7$  cGy/h. As seen previously with the acute X-ray response, the data for  $6.7$  and  $14.3$  cGy/h were best fit by linear–quadratic



(a)



(b)

Figure 5(A). Comparison of mutation versus survival in WTK1 at different dose-rates. Points are the average survival plotted against the average mutation induction for each dose, and use the same symbols as Figure 3(A). (B) Comparison of mutation versus survival in TK6 at different dose-rates. Points are the average survival plotted against the average mutation induction for each dose, and use the same symbols as Figure 3(B).

curves. The response at  $2.7$  cGy/h was the most linear, but fit equally well to a linear quadratic curve. The spontaneous *hprt* mutation rate observed in WTK1 was  $0.51 \times 10^{-6}$ /cell/generation through the course of the long-term exposures.

In TK6 the  $14.3$  cGy/h dose-rate decreased the yield of *hprt* mutants marginally but not significantly ( $p > 0.1$ ), while at  $6.7$  and  $2.7$  cGy/h the induction of mutants was statistically greater ( $p > 0.001$ ) than that observed for acute irradiation when the slopes of the induction curves were compared (Figure 4(B)). During the long-term exposures the spontaneous mutation rate of TK6 was measured as  $0.18 \times 10^{-6}$ /cell/generation in the control cultures. Interestingly, at the lowest dose-rate ( $2.7$  cGy/h), the dose–response for *hprt* mutant induction was essentially identical in TK6

and WTK1. When a linear fit was used for mutant induction in WTK1, the slopes were not different ( $p > 0.1$ ).

When mutation induction was plotted against survival, none of the dose-rates had a differential effect on WTK1 (Figure 5(A)). However, in TK6 (Figure 5(B)) 6.7 and 2.7 cGy/h were considerably more efficient than acute exposures at mutation per lethal event due to the dose-rate effect on survival being more pronounced than the effect on mutation. However, the mutation per survivor relationship was not different for acute irradiation and 14.3 cGy/h in TK6.

#### 4. Discussion

Previous studies of dose-rate in TK6 concluded that there was no dose-rate effect on mutation in these cells. Grosovsky and Little (1985) used X-ray fractions of 1–10 cGy/day, which would fall significantly below the dose-rates employed in the present study; our lowest dose-rate would result in an accumulated dose of 64.8 cGy/day. Liber *et al.* (1985) employed tritiated water to produce continuous  $\beta$ -irradiation with dose-rates between 0.324 and 3.8 cGy/h. Within this range of dose-rates, no differences in dose–response of mutation induction were seen. However, the induction of mutants per cGy was higher by the low dose-rate  $\beta$ -particles than by acute X-ray exposure. These results may be explained in part by the differences in radiations, as  $\beta$ -particles have been reported to be somewhat more efficient at mutant-induction than  $\gamma$ -rays per Gy (Ueno *et al.* 1982).

Continuous low dose-rate exposure to  $\gamma$ -rays (from  $^{60}\text{Co}$ ) has been employed in only one previous study with TK6 cells (König and Kiefer 1988), which reported no difference between mutation induction by acute X-ray exposures and either 0.27 or 2.7 cGy/h  $^{60}\text{Co}$   $\gamma$ -rays. Although our data indicate significant differences between acute exposure and both 2.7 and 6.7 cGy/h (Figure 4(B)), the differences in induced mutant fractions are small within the range of the acute doses. The reduced cytotoxicity of the lower dose-rates made it feasible to measure mutation induction at much higher doses than is possible with acute exposures. As a result, the statistical comparisons may be somewhat skewed by inclusion of data from higher doses in the low dose-rate data sets than those included in the acute exposure data. It should also be noted that König and Kiefer (1988) used acute X-ray exposures to compare with their low dose-rate  $^{60}\text{Co}$   $\gamma$ -ray

exposures. In our present study, we have used acute exposure to  $^{137}\text{Cs}$   $\gamma$ -rays for all our comparisons, and the rate of *hprt* mutant induction was somewhat lower than we found in our previous acute exposure studies with X-rays. Compared with previously reported X-ray mutation-induction data (Liber *et al.* 1983, Amundson and Liber 1991) the induction of mutants by our 2.7 cGy/h  $^{137}\text{Cs}$  exposure was not significantly different ( $p > 0.1$ ;  $0.1 > p > 0.05$  respectively). Any real differences in mutant induction by acute and low dose-rate exposures in TK6 would appear so slight they may easily vanish in the ‘noise’ of the system. In this case, comparison with the appropriate acute radiation exposure may be important to the interpretation of low dose-rate studies.

In each of the previous studies, induction of thymidine kinase (*tk*) mutants was also measured and reported to be independent of dose-rate. These studies were performed before the discovery of the class of slowly growing *tk* mutants (Yandell *et al.* 1986), which are now known to comprise the majority of *tk* mutants in TK6. WTK1 does not show the same proportions of normally and slowly growing *tk* mutants, but a majority of the mutants induced by acute X-ray exposure do show at least a transient period of slow growth (Amundson *et al.* 1993). For these reasons, a comparison of *tk* mutant induction in long-term low dose-rate experiments would not be practical in these cell lines. The different growth rates of mutants would require too many assumptions for meaningful interpretation of mutant induction during protracted exposures. This is somewhat unfortunate, because in previous studies the difference in mutability between TK6 and WTK1 was much more pronounced at the *tk* locus than at *hprt*. As no such discrepancies in growth rates have ever been detected in *hprt* mutants, however, this locus is an appropriate endpoint for low dose-rate studies.

The difference between TK6 and WTK1 survival following acute X-irradiation is not as pronounced as that generally observed between other radiation-sensitive mutants and their more resistant parents. Also, both cell lines in the current study show a dose-rate effect for killing. In most DNA double-strand break (dsb) repair-deficient mutants survival is independent of the dose-rate. Our results indicate that there is some repair of prelethal lesions (or ‘sublethal damage’) in TK6, but that this process may be accomplished more effectively in WTK1. This interpretation is consistent with the results of our recent comparison on DNA dsb repair capacities in these two cell lines

(manuscript in preparation) which showed that TK6 leaves more unrepaired lesions than WTK1 following exposure to high doses of X-rays. An earlier study also indicated that TK6 was deficient in the repair of double-strand breaks measured by elution (Evans *et al.* 1993). WTK1 has now also been shown to repair a higher percent of lesions than TK6 in the same assay (H. Evans, personal communication).

The survival curve of TK6 with 2.7 cGy/h irradiation shows a plateau effect after an initial sharp decrease during the first few days of exposure. This could indicate either the selection of a pre-existing radiation resistant subpopulation of TK6 cells, or the induction of DNA repair. In order to test the possibility that the low dose-rate irradiations selected for resistant cells, clones surviving maximum irradiation at 2.7 cGy/h were tested for survival to acute  $\gamma$ -ray exposures. All clones tested had  $D_0$  values from 70–75 cGy compared to 80 cGy for the original TK6. As the cloning and expansion of the cultures following irradiation required several weeks, survival of the previously irradiated (non-clonal) TK6 populations was also examined in case the cloning process allowed time for the loss of a transient enhancement in radiation resistance. The previously irradiated cultures were consistently slightly more sensitive to acute irradiation, perhaps because they had not completely recovered from the prior exposure. Hence, the low dose-rate exposures did not select for the survival of a resistant subpopulation in TK6, and no long-lived 'radioadaptive' response was detectable. However, it is still possible that persistent low dose-rate irradiation of TK6 may induce a transient increase in DNA repair. This process may only be efficient at 2.7 cGy/h where damage to the population accumulates more slowly than at the higher dose-rates.

In previous analyses, comparisons of mutation induction per lethal event have been used to clarify the responses of the radiation-sensitive cell lines. In general, the radiation-sensitive variants have been rodent cell lines, that tend to be hypermutable compared with their radiation resistant parents. In these cases, the sensitive and resistant cell lines give survival versus mutation plots with the same slope, indicating that it simply requires a lower dose to elicit the same overall effect in the more sensitive variants. Since the relatively radiation sensitive TK6 is hypomutable compared to WTK1, such a plot actually exaggerates the differences between these lines rather than equalizing them. Similarly, the dose-rate effects on a cell line can be equalized by comparing survival versus mutation (e.g. Thacker and Stretch 1983). This

indicates that protraction of the dose has a similar magnitude of effect on both endpoints. This is what is seen in WTK1. In TK6, however, the dose-rate effects on survival do not parallel the effects on mutation. While acute exposures and 14.3 cGy/h have similar slopes, 6.7 and 2.7 cGy/h exposures give greater induction of mutation at the same amount of cell killing. Whether or not prelethal and premutagenic lesions are different, the separation of the mutation and survival responses in TK6 seems to indicate that there are different mechanisms for damage processing. TK6 would appear to have a defect in at least one of these pathways. Hence, the effects observed with TK6 are likely to result from the specific biochemical defect in TK6 rather than to a general difference between human and rodent cell lines.

WTK1 has been shown to have a homozygous mutation in the p53 gene, whereas TK6 is homozygous wide-type at this locus (Carrier *et al.* 1994, Xia *et al.* 1995). The mutant protein accumulates to high levels in WTK1 cells, but maintains a wild-type conformation and DNA binding activities (Carrier *et al.*, in press). Although it is unlikely that all the differences we have observed between these cell lines can be attributed to the mutation in p53, mutant p53 can contribute to enhanced radiation survival (Lee and Bernstein 1993, Fan *et al.* 1994). A change in the activity of p53 may occur in WTK1, as Xia *et al.* (1995) have reported that the mutant p53 in these cells was associated with a delayed apoptotic response following X-irradiation. Apoptosis did, however, ultimately reach the same level in both cell lines. If apoptosis occurs more rapidly, or at lower doses in TK6, a higher proportion of TK6 cells may undergo apoptosis during low dose-rate exposures. More efficient removal of the most damaged cells from the population might then contribute to the lower overall induction of mutants in TK6 compared with WTK1.

The inversion of the dose-rate effect for neoplastic transformation is of considerable interest because of the implications for human exposures. A possible mechanism for this effect postulates the passage of cells through a narrow 'window' of sensitivity in the cell cycle (Rossi and Kellerer 1986, Brenner and Hall 1990, Elkind 1991). At lower dose-rates, less induction of cell-cycle delay and less killing, allow more cells to continue moving through the highly sensitive phase, thus increasing the number of cells at high risk, and the ultimate transformation frequency. Higher dose-rates would induce more cell-cycle delay and more killing, thus decreasing the number of cells that

would progress through the high risk phase during the period of irradiation. Although this mechanism could not account for the differences in mutation observed between the cell lines, it could be involved in the dose-rate effect produced in both lines, because the cells continued to cycle throughout the exposure period.

Different cell-cycle phase-specific sensitivities to radiation mutation induction have been reported for several cell lines (Arlett and Potter 1971, Watanabe and Horikawa 1977, Burki 1980), although no general pattern of sensitivity has emerged. Cell-cycle dependent mutability of TK6 cells has been studied with chemical mutagens (Hoppe *et al.* 1991) and recently X-ray induction of mutants in lovastatin synchronized WTK1 cells has been investigated (H. Liber, personal communication). Early G<sub>1</sub> seems the most sensitive for *hprt* mutant induction, with G<sub>1</sub> overall 1.5–2-fold more sensitive than G<sub>2</sub>. In the present experiments, however, no consistent trend of cell cycle redistribution is evident in either cell line (Figures 2(A) and 2(B)), and the transient increase of cells in G<sub>2</sub> is not reflected by a corresponding reduction in mutation efficiency. Rather than an accumulation of cells in a more resistant cell-cycle phase or ‘window’ at the higher low dose-rates, it seems likely that the more rapid and regular cycling of cells through sensitive portions of the cell cycle (such as early G<sub>1</sub>) accounts for the enhanced effects of the lower low dose-rates. Ultimately, the balance between apoptosis and population redistribution, and their interaction with cellular repair capabilities may shape the mutational response at different rates of exposure.

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## References

AMUNDSON, S. A. and LIBER, H. L., 1991, A comparison of induced mutation at homologous alleles of the tk locus in human cells. *Mutation Research*, **247**, 19–27.

- AMUNDSON, S. A., XIA, F., WOLFSON, K. B. and LIBER, H. L., 1993, Different cytotoxic and mutagenic responses induced by X-rays in two human lymphoblastoid cell lines derived from a single donor. *Mutation Research*, **286**, 233–241.
- ARLETT, C. F. and POTTER, J., 1971, Mutation to 8-azaguanine resistance induced by gamma radiation in a Chinese hamster cell line. *Mutation Research*, **13**, 59–65.
- BENJAMIN, M. B., POTTER, H., YANDELL, D. W. and LITTLE, J. B., 1991, A system for assaying homologous recombination at the endogenous human thymidine kinase gene. *Proceedings of the National Academy of Science, USA*, **88**, 6652–6656.
- BRENNER, D. J. and HALL, E. J., 1990, The inverse dose rate effect for oncogenic transformation by neutrons and charged particles. A plausible interpretation consistent with published data. *International Journal of Radiation Biology*, **58**, 745–758.
- BURKI, H. J., 1980, Ionizing radiation-induced 6-thioguanine-resistant clones in synchronous CHO cells. *Radiation Research*, **81**, 76–84.
- CARRIER, F., BAE, I., SMITH, M. L., AYERS, D. A. and FORNACE, A. J. JR., 1996, Characterization of the GADD45 response to ionizing radiation in WI-L2-NS cells, a p53 mutant cell line. *Mutation Research* (in press).
- CARRIER, F., SMITH, M. L., BAE, I., KILPATRICK, K. E., LANSING, T. J., CHEN, C. -Y., ENGELSTEIN, M., FRIEND, S. H., HENNER, H. D., GILMER, T. M., KASTAN, M. B. and FORNACE, A. J. JR., 1994, Characterization of human gadd45, a p53-regulated protein. *Journal of Biological Chemistry*, **269**(51), 32672–32677.
- CRISSMAN, H. A. and STEINKAMP, J. A., 1982, Rapid, one step staining procedures for analysis of cellular DNA and protein by single and dual laser flow cytometry. *Cytometry*, **3**, 84–90.
- CROMPTON, N. E. A., BARTH, B. and KIEFER, J., 1990, Inverse dose-rate effect for the induction of 6-thioguanine-resistant mutants in Chinese hamster V79-S cells by <sup>60</sup>Co  $\gamma$ -rays. *Radiation Research*, **124**, 300–308.
- ELKIND, M. M., 1991, Enhanced neoplastic transformation due to protracted exposures of fission-spectrum neutrons: a biophysical model. *International Journal of Radiation Biology*, **59**, 1467–1475.
- ELKIND, M. M. and SUTTON, H., 1959, X-ray damage and recovery in mammalian cells in culture. *Nature*, **184**, 1293–1295.
- ELKIND, M. M., SUTTON-GILBERT, H., MOSES, W. B. and KAMPER, C., 1967, Sub-lethal and lethal radiation damage. *Nature*, **214**, 1088–1090.
- EVANS, H. H., HORNG, M. R., MENCL, J., GLAZIER, K. G. and BEER, J. Z., 1985, The influence of dose rate on the lethal and mutagenic effects of X-rays in proliferating L5178Y cells differing in radiation sensitivity. *International Journal of Radiation Biology*, **47**, 553–562.
- EVANS, H. H., NIELSEN, M., MENCL, J., HORNG, M. F. and RICANATI, M., 1990, The effect of dose rate on X-radiation-induced mutant frequency and the nature of DNA lesions in mouse lymphoma L5178Y cells. *Radiation Research*, **122**, 316–325.
- EVANS, H. H., RICANATI, M., HORNG, M. F., JIANG, Q. and OLIVE, P., 1993, DNA double-strand break rejoining deficiency in TK6 and other human B-lymphoblast cell lines. *Radiation Research*, **134**, 307–315.
- FAN, S., EL-DEIRY, W., BAE, I., FREEMAN, J., JONDLE, D., BHATIA, K., FORNACE, A. J. FR., MAGRATH, I., KOHN, K. W. and O’CONNOR, P. M., 1994, p53 gene mutations are associated with

- decreased sensitivity of human lymphoma cells to DNA damaging agents. *Cancer Research*, **54**, 5824–5830.
- FURTH, E. E., THILLY, W. G., PENMAN, B.W., LIBER, H. L. and RAND, W. M., 1981, Quantitative assay for mutation in diploid human lymphoblasts using microtiter plates. *Analytical Biochemistry*, **110**, 1–8.
- FURUNO-FUKUSHI, I., UENO, A. M. and MATSUDAIRA, H., 1988, Mutation induction by very low dose-rate  $\gamma$ -rays in cultured mouse leukemia cells L5178Y. *Radiation Research*, **115**, 273–280.
- GROSOVSKY A. J. and LITTLE, J. B., 1985, Evidence for linear response for the induction of mutations in human cells by x-ray exposures below 10 rads. *Proceedings of the National Academy of Sciences, USA*, **82**, 2092–2095.
- HOPPE, H. IV., CALL, K. M., LEONG, P. -M, and THILLY, W. G., 1991, Cell-cycle dependent mutation of human lymphoblasts: Bromodeoxyuridine and butyl methane-sulfonate. *Mutation Research*, **250**, 411–421.
- KÖNIG, F. and KIEFER, J., 1988, Lack of dose-rate effect for mutation induction by  $\gamma$ -rays in human TK6 cells. *International Journal of Radiation Biology*, **54**, 891–897.
- LEE, J. M. and BERNSTEIN, A., 1993, p53 mutations increase resistance to ionizing radiation. *Proceedings of the National Academy of Sciences, USA*, **90**, 5742–5746.
- LEVY, J. L., VIROLAINEN, M. and DEFENDI, V., 1968, Human lymphoblast lines from lymph node and spleen. *Cancer*, **22**, 517–524.
- LIBER, H. L., LEMOTTE, P. K. and LITTLE, J. B., 1983, Toxicity and mutagenicity of X-rays and [<sup>125</sup>I]dUrd or [<sup>3</sup>H]TdR incorporated in the DNA of human lymphoblast cells. *Mutation Research*, **111**, 387–404.
- LIBER, H. L., OZAKI, V. H. and LITTLE, J. B., 1985, Toxicity and mutagenicity of low dose rates of ionizing radiation from tritiated water in human lymphoblastoid cells. *Mutation Research*, **157**, 77–86.
- LIBER, H. L. and THILLY, W. G., 1982, Mutation assay at the thymidine kinase locus in diploid human lymphoblasts. *Mutation Research*, **94**, 467–485.
- LORENZ, R., DEUBEL, W., LEUNER, K., GÖLLNER, T., HOCHHAUSER, E. and HEMPEL, K., 1994, Dose and dose-rate dependence of the frequency of HPRT deficient T lymphocytes in the spleen of the <sup>137</sup>Cs gamma-irradiated mouse. *International Journal of Radiation Biology*, **66**, 319–326.
- LORENZ R., LEUNER, K., DEUBEL, W., GÖLLNER, T. and HEMPEL, K., 1993, Normal and reverse dose-rate effect for the induction of mutants in somatic cells by ionizing radiation. *Toxicology Letters*, **67**, 353–363.
- NAGASAWA, H. N., CHEN, D. J. and STRNISTE, G. F., 1989, Response of X-ray-sensitive CHO mutant cells to  $\gamma$ -radiation. I. Effects of low dose rates and the process of repair of potentially lethal damage in G<sub>1</sub> phase. *Radiation Research*, **118**, 559–567.
- NAGASAWA, H. N., LITTLE, J. B., TSANG, N. M., SAUNDERS, E., TESMER, J. and STRNISTE, G. F., 1992, Effect of dose rate on the survival of irradiated human skin fibroblasts. *Radiation Research*, **132**, 375–379.
- ONG, A., LI, W. X. and LING, C. C., 1993, Low-dose-rate irradiation of rat embryo cells containing the *Ha-ras* oncogene. *Radiation Research*, **134**, 251–255.
- ROSSI, H. H. and KELLERER, A. M., 1986, The dose rate dependence of oncogenic transformation by neutrons may be due to variation of response during the cell cycle. *International Journal of Radiation Biology*, **50**, 353–361.
- SKOPEK, T. R., LIBER, H. L., PENMAN, B. W. and THILLY, W. G., 1987, Isolation of a human lymphoblastoid line heterozygous at the thymidine kinase locus: possibility for a rapid human cell mutation assay. *Biochemical and Biophysical Research Communications*, **84**, 411–416.
- STACKHOUSE, M. A. and BEDFORD, J. S., 1993, An ionizing radiation-sensitive mutant of CHO cells: *ixs-20*. II. Dose-rate effects and cellular recovery processes. *Radiation Research*, **136**, 250–254.
- THILLY, W. G., DELUCA, J. G., FURTH, E. E., HOPPE, H. IV., KADEN, D. A., KROLEWSKI, J. J., LIBER, H. L., SKOPEK, T. R., SLAPIKOFF, S. A., TIZARD, R. J. and PEMAN, B. W., 1980, Gene locus mutation assays in diploid human lymphoblast lines, in: *Chemical Mutagens*, vol. 6, Edited by F. J. DeSerres and A. Hollaender, (Plenum, New York), pp. 331–364.
- THACKER, J. and STRETCH, A., 1983, Recovery from lethal and mutagenic damage during postirradiation holding and low-dose-rate irradiations of cultured hamster cells. *Radiation Research*, **96**, 380–392.
- UENO, A. M., FURUNO-FUKUSHI, I. and MATSUDAIRA, H., 1982, Induction of cell killing, micronuclei, and mutation to 6-thioguanine resistance after exposure to low-dose-rate  $\gamma$ -rays and tritiated water in cultured mammalian cells (L5178Y). *Radiation Research*, **91**, 447–456.
- WATANABE, M. and HORIKAWA, M., 1977, Analyses of differential sensitivities of synchronized HeLa S3 cells to radiation and chemical carcinogens during the cell cycle. IV. X-rays. *Mutation Research*, **44**, 413–426.
- XIA, F., AMUNDSON, S. A., NICKOLOFF, J. A. and LIBER, H. L., 1994, Different capacities for recombination in closely related human lymphoblastoid cell lines with different mutational responses to X-irradiation. *Molecular and Cellular Biology*, **14**, 5850–5857.
- XIA, F., WANG, X., WANG, Y. H., TSANG, N. M., YANDELL, D. W., KELSEY, K. T. and LIBER, H. L., 1995, Altered p53 status correlates with differences in sensitivity to radiation-induced mutation and apoptosis in two closely related human lymphoblast lines. *Cancer Research*, **55**, 12–15.
- YANDELL, D. W., DRYJA, T. P. and LITTLE, J. B., 1986, Somatic mutations at a heterozygous autosomal locus in human cells occur more frequently by allele loss than by intragenic structural alterations. *Somatic Cell Molecular Genetics*, **12**, 255–263.
- ZEMAN, E. M. and BEDFORD, J. S., 1985, Dose fractionation effects in plateau-phase cultures of C3H10T $\frac{1}{2}$  cells and their transformed counterparts. *Radiation Research*, **101**, 373–393.