LA-UR 94-4389

CONF-9407154--2

Los Alamos National Laboratory is operated by the University of California for the United States Department of Energy under contract W-7405-ENG-36.

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SUBMITTED TO: Advances in Space Research

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FORM NO 836 R4
ST NO. 2629 5/81

IONIZING RADIATION-INDUCED MUTATION OF HUMAN CELLS WITH DIFFERENT DNA REPAIR CAPACITIES

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ABSTRACT

We have observed significant differences in the response to ionizing radiation of two closely related human cell lines, and now compare the effects on these lines of both low and intermediate LET radiation. Compared to TK6, WTK1 has an enhanced X-ray survival, and is also more resistant to cell killing by α-particles. The *hprt* locus is more mutable in WTK1 than in TK6 by both X-rays and α-particles. WTK1 is also more mutable by α-particles than by X-rays at the *hprt* locus. X-ray-induced mutation at the heterozygous *tk* locus in WTK1 is about 25 fold higher than in TK6, while α-particle-induced mutation is nearly 50 fold higher at this locus. Also, the slowly growing *tk*- mutants, which comprise the majority of spontaneous and X-ray-induced *tk*- mutants of TK6, were not induced significantly by α-particles. Previously, we showed that TK6 has a reduced capacity for recombination compared with WTK1, and therefore, these results indicate that recombinational repair may contribute to both cell survival and mutation-induction following exposure to ionizing radiation. Such a mechanism may aid cell survival, but could also result in increased deleterious effects such as the unmasking of recessive mutations in cancer suppresser genes.

INTRODUCTION

The study of the biological effects of high LET irradiation is important to the estimates of risk to those exposed to such radiations either in space or on earth. The initial damage induced in cells by high LET radiations seems to differ from that induced by low LET such as X-rays /1,2,3/. The repair of high LET induced cellular damage may also be less efficient than that of low LET damage /4,5,6,7/. Since there does not appear to be a simple relationship between LET and the biological endpoints measured in cultured cells, it is necessary to study specific particles which may have relevance to human exposures and disease.

Radon, a predominant α-particle emitter, has been definitively linked to the causation of lung cancers /8,9/ and possibly leukaemias /10,11/. Because relatively high levels of radon and radon daughters are present in many homes, the mechanisms by which

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α-emitters may pose a danger to human health must be examined more precisely. Our approach to this problem has been to pursue a better understanding of the cellular and molecular responses of human cells exposed to α-particles. The cytotoxic and mutagenic effects of α-particles have been examined in several hamster cell lines /12, 13,14,15/, mouse lymphoma cells, /16,17/ and human fibroblasts /18/. We now present a study of α-particle effects on two human lymphoblast cell lines.

The TK6 cell line is extremely well suited to mutation studies. Many reports on the chemical and X-ray induction of mutations at the hypoxanthine-guanine phosphoribosyl transferase (hprt) /19,20/, thymidine kinase (tk) /20,21,22/, adenine phosphoribosyl transferase (aprt) /23,24/, and other loci have validated the use of these cells. High LET effects on TK6 previously have been examined by the incorporation of ^{125}I /25, 26/, bombardment with ^{28}Si and ^{40}Ar ions /27/, neutrons /28/ and chelated ^{212}Bi in solution /29/.

More recently, we have described several cell lines, including WTK1, which are closely related to TK6. These cell lines are less sensitive to cell killing, but more mutable following exposure to X-rays /30/. The observed effects may be due, at least in part, to a higher capacity of WTK1 cells to catalyze recombination as assayed in a plasmid based system, and as evidenced by molecular analysis of tk- mutants /31/. WKT1 also has a higher repair capacity for X-ray-induced double strand breaks than has TK6 (unpublished results.) These two cell lines represent a well characterized and unique system for the comparison of the effects DNA damaging agents on human cells with different capacities for recombination and dsb repair. We have irradiated these two cell lines with α-particles and compared the survival and induced mutation at the hprt and tk loci with that induced by X-irradiation.

MATERIALS AND METHODS

Cell Lines

WIL2 is a nonclonal isolate from a human spleen first described by Levy et al. /32/. This culture was widely distributed and has been used in many different laboratories. WI-L2-NS (ATCC CRL 8155) is a subclone of WIL2 which was later deposited at the ATCC. A different unselected clone, HH4, was used to derive the TK6 cell line, which is heterozygous for the thymidine kinase (tk) gene /33/. WTK1, a *tk* heterozygote derived from the WIL2-NS cell line, was obtained from M.B. Benjamin. (Figure 1). A *Sac*I polymorphism distinguishes the two alleles of the *tk* gene in WIL2-NS and TK6 cells /34/.

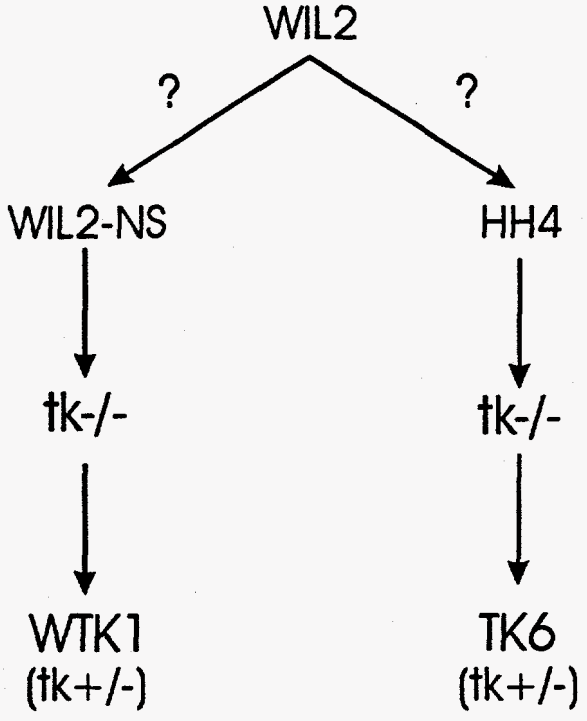

Fig. 1 Relationship between the cell lines used in these studies.

Cells were maintained as exponentially growing cultures in RPMI 1640 medium supplemented with 10% horse serum (heat treated for 2 hours at 56°C). The cultures were incubated at 37°C in 5% CO_2 and 100% humidity and maintained at densities of 1-12 x 10^5 cells/ml.

Irradiations

Prior to the start of mutation experiments, CHAT (deoxycytidine, hypoxanthine, aminopterin, and thymidine) treatment of cultures was carried out as previously described /20/. X-ray irradiations were performed with a Philips MG-102 X-ray generator operating at 9.6 mA with 1 mm Al added filtration. The dose-rate to the cells was approximately 76 cGy/min, as determined with a Victoreen ionization chamber and thermoluminescent dosimetry.

The alpha particle source used for these experiments has been described in detail elsewhere /35/, and consists of a thin layer of ^{238}Pu electrodeposited onto a stainless steel disk. The beam passes through an aluminum collimator, and exposure times are controlled by means of a photographic shutter. Due to the high elevation of Los Alamos (7300 feet above sea level) air pressure is approximately 30% lower than that at sea level. This results in sufficiently low attenuation of the alpha particles to allow irradiation in atmosphere, while retaining an acceptable width to the energy spectrum. At the cell mylar interface, the dose rate is approximately 3.8 cGy/sec with a mean energy of 3.5 MeV and LET of 116 keV/μm.

Lymphoblasts growing in suspension culture were pelleted by centrifugation and pipetted directly onto the 1.5 μm thick mylar bottomed dishes especially constructed for use with this alpha source. The cells where then covered with a glass coverslip to force them into a "monolayer" on the mylar. Microscopic examination indicated that all cells were in contact with the mylar base of the dish. A similar technique has been described for irradiating bone marrow cells /36/.

Immediately after irradiation, cultures were plated for survival in 96-well microtiter plates at between 1 and 100 cells/well. Following appropriate expression times, cells were plated in 0.5 μg/ml 6-thioguanine to select for hprt- mutants, or 2.0 μg/ml trifluorothymidine to select for tk- mutants. Plates were incubated and scored for colony formation after 11 days, at which time fresh trifluorothymidine was added to the tk- plates in order to score for late appearing colonies. Mutant fractions were calculated using the method of Furth *et al.* /37/.

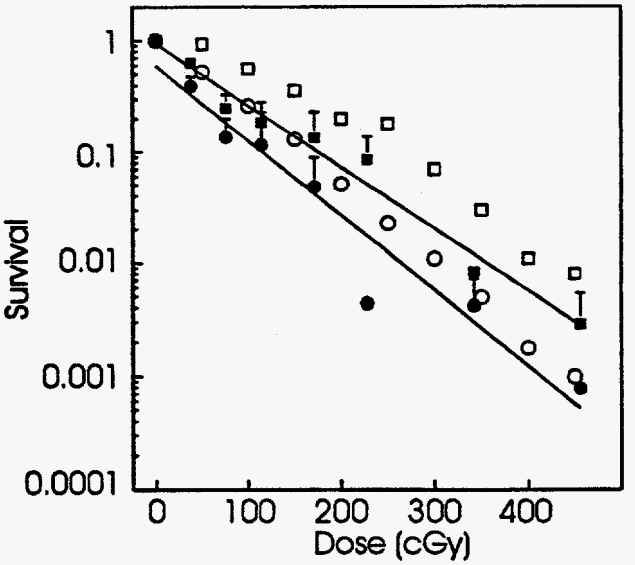

Fig. 2 Survival of the TK6 (circles) and WTK1 (squares) following X-ray (open) and alpha (closed). Error bars are SEM.

RESULTS AND DISCUSSION

Both the cell lines used in this study were more susceptible to killing by α-particles than by X-rays. This difference was slightly more pronounced in TK6 (RBE 1.7) than in WTK1 (RBE 1.3). ^{212}Bi α-particle irradiation of TK6 cells was reported to have a somewhat higher RBE of 3.5 /29/. WTK1 ($D_0 \approx 73$ cGy) also has higher survival than TK6 ($D_0 \approx 40$ cGy) following α-particle irradiation (Figure 2). This result is in agreement with other studies using the same alpha source which demonstrated that cell lines with lower survival following γ-ray exposure also had lower survival after α-particle irradiations /13/. However, Evans et al., /17/ reported that while mutants of mouse L5178Y cells with differing DNA repair capacities exhibited a range of X-ray sensitivities their sensitivity to α-particles was the same. This may imply that these mutants process X-ray and α-particle damage by different DNA repair pathways.

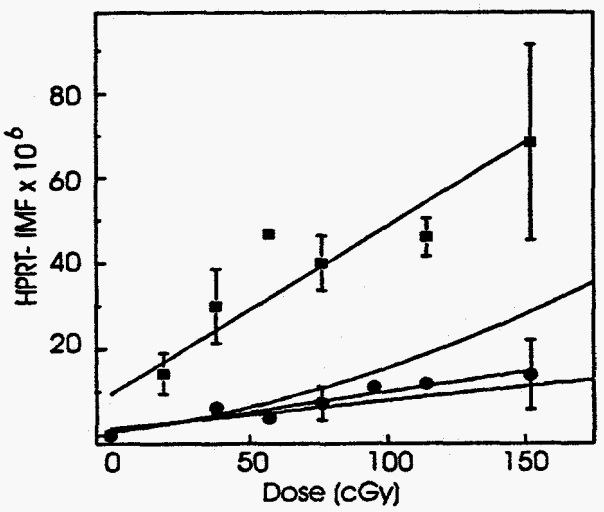

Fig. 3 α-particle-induced hprt- mutation: TK6 (circles) and WTK1 (squares). Lines only for X-ray induced mutants. Error bars are SEM.

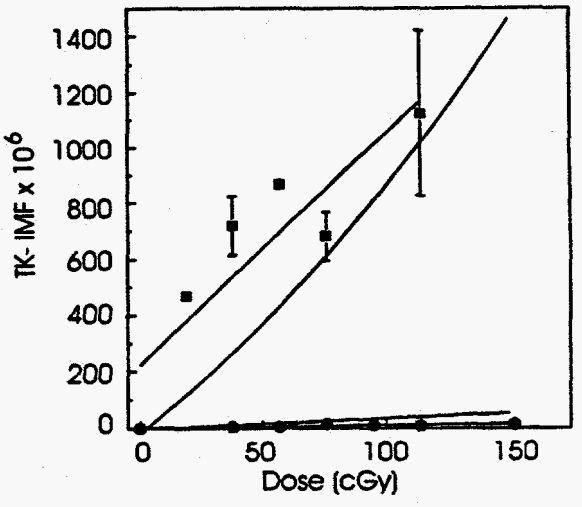

Fig. 4 α-particle-induced tk- mutation in TK6 (circles) and WTK1 (squares). Lines only for X-ray-induced mutants. Error bars are SEM.

Alpha particles induced mutations at the hprt locus in WTK1 with higher efficiency than did X-rays (Figure 3). The data for X-rays is fit best by a linear quadratic dose response while the induction of mutants by α-particles can be fit by a linear regression. A greater induction of hprt mutants by α-particles compared to X-rays was previously reported in human fibroblasts /18/ Conversely, the induction of hprt mutants in TK6 by both X-rays and α-particles was linear and of approximately equal efficiency. Therefore, the hprt locus is approximately 4-fold more mutable by α-particles in WTK1 than it is in TK6. In a previous study, TK6 did show a 3.8-fold higher mutability by ^{212}Bi than X-rays at the hprt locus /29/. This difference may be related to the higher energy alpha particles emitted by ^{212}Bi, or to the differences in dosimetry or exposure methods.

The α-particle-induced mutant fractions at the autosomal heterozygous tk locus are shown compared to the X-ray induced mutant fractions in Figure 4. In all cases, only total tk- mutant fractions are shown, which are the sum of early and late appearing mutants previously described /22/. WTK1 is at least 50-fold more mutable at the tk

locus by α-particles than is TK6. WTK1 is also about 3-fold more mutable per cGy by α-particles than by X-rays. TK6, however, is about 3-fold less mutable by α-particles than by X-rays at the tk locus. This finding is similar to that of Metting et al /29/ with ^{212}Bi which reported an RBE of 0.83 for all tk mutants.

The majority of X-ray and spontaneous tk- mutants of TK6 have been found to have a stable slow growth rate in culture. Although around 80% of tk mutants of WTK1 also appear only after 18 days of incubation, less than half of these exhibit a stable slow growth phenotype. A similar pattern was seen among α-particle-induced mutants of both TK6 and WTK1. This is in agreement with the findings of Metting *et al.* /29/ with ^{212}Bi in TK6, where 9/9 late arising tk- clones were found to have normal (<20 hour) doubling times when tested. The low induction of tk- mutants of TK6, and the reduction in the proportion of these mutants with longer than normal doubling times may indicate a different mechanism is responsible for the induction of mutants by α-particles than by X-rays. X-ray-induced tk- mutants of WTK1 have been associated predominantly with large scale gene conversion or genetic recombination, while mutants of TK6 were due mainly to deletion of the active allele /31/.

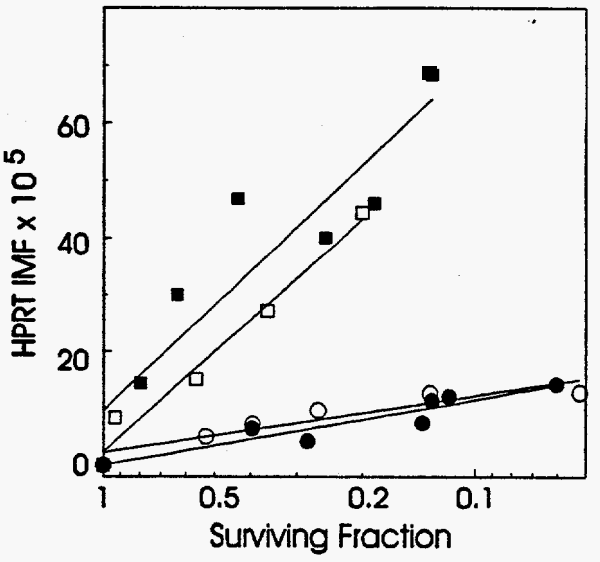

Fig. 5 Induced hprt mutation as a function of survival for TK6 (circles) and WTK1 (squares) irradiated with X-rays (open) or α-particles (filled).

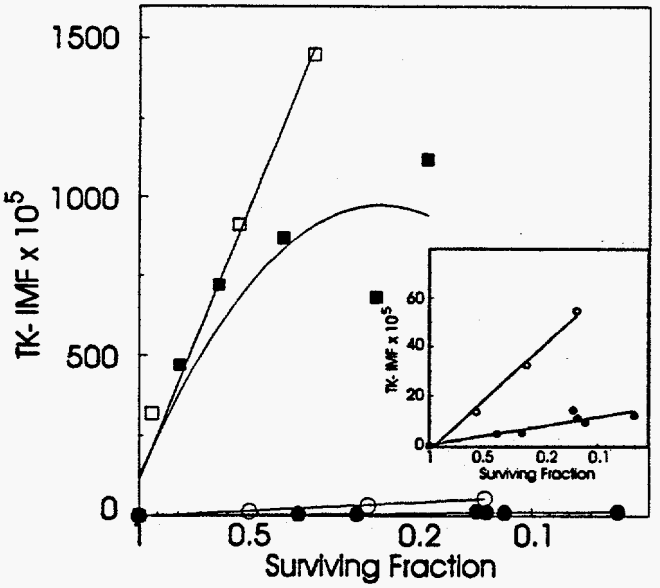

Fig. 6 Induced tk- mutation as a function of survival for TK6(circles), and WTK1 (squares) irradiated with X-rays (open) or α-particles (closed).

It is possible that gene conversion events are much more efficient for the processing of α-particle-induced damage, and that even in the recombination deficient cell line TK6, a higher proportion of the recoverable events are due to such a mechanism. This could also account for the reduced efficiency of mutation induction by α-particles compared to X-rays which is seen at the tk locus in TK6, but not in WTK1. Also, as such a mechanism would not be expected to play a major role at the hemizygous hprt locus, this is consistent with the similar efficiencies of the two radiations at this locus in TK6.

Comparisons of relative mutation induction versus survival at the same dose have been used to clarify the relationships between different irradiation conditions or

between DNA repair deficient mutants and their repair proficient parent lines. The induced mutant fractions at the hprt and tk loci in the two cell lines used here are shown in Figures 5 and 6 as a function of survival. For hprt, the mutants per survivor relationship is similar for the two radiations in both the cell lines. However, there is a significant difference between the two cell lines. Similarly for the tk locus, the greatest difference in this parameter is between the cell lines. The relationship is similar for X-rays and alpha particles at lower doses in WTK1, but the efficiency of mutation by α-particles falls off at higher doses. For TK6, however, X-rays are notably more efficient at inducing tk- mutation at all survival levels than are α-particles. A similar higher efficiency of α-particles over X-rays has been reported for the hprt locus in Chinese hamster V79 cells /12/ and human fibroblasts /18/. This demonstrates that the reduction in the recovery of tk- mutants from α-irradiated TK6 cells can not be explained by the differences in survival, and indicates a real difference in the mechanisms of mutation which operate efficiently following different types of radiation damage in these cells.

ACKNOWLEDGMENTS

We would like to thank M.R. Raju for helpful discussions and Richard T. Okinaka for critical reading of the manuscript. This work was supported by the U.S. Dept. of Energy.

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