RADIATION RESEARCH **99,** 52–58 (1984)

The Mutagenicity of Alpha Particles in Ehrlich Ascites Tumor Cells

GEORGE ILIAKIS[1]

Institut fuer Biophysik der Universitaet und Gesellschaft fuer Strahlen- und Umweltforschung, Paul-Ehrlich-Strasse 20 D-6000 Frankfurt/Main, West Germany

ILIAKIS, G. The Mutagenicity of Alpha Particles in Ehrlich Ascites Tumor Cells. *Radiat. Res.* **99,** 52–58 (1984).

Cell killing and the induction of mutation to thioguanine resistance (HGPRT enzyme deficiency) were measured after exposure of Ehrlich ascites tumor cells to 150-kV X rays and ^{241}Am α particles. The curve describing the induction of mutations was almost linear after exposure to α particles (slope: 14.1×10^{-5} Gy^{-1}) but upward bending after exposure to X rays, apparently reaching a final slope similar to that obtained after exposure to α particles. The number of mutants induced per viable cell by α particles at a given level of cell killing was similar to that induced by X rays. The RBE values obtained for cell killing and the induction of mutations are compared with each other, and the possible involvement of repair processes in determining the RBE is discussed.

INTRODUCTION

It is well established that both cell inactivation and the induction of mutations in mammalian cells depend upon the linear energy transfer (LET) of the radiation used. High-LET radiations were found to be more effective in inactivating cells, with a maximum at values between 100 and 200 keV/μm (*1–3*). Similar results were also obtained for the induction of mutations by radiations of various LET with a maximum also in the range between 100 and 200 keV/μm (*4–6*).

The RBE values observed, both for cell inactivation and for mutation induction, after exposure to high-LET radiations vary significantly from one biological system to another and appear also to depend on the end point measured. Higher RBE values for mutation induction than for cell inactivation have been observed after exposure of V79-4 hamster and human cells to radiations of various LET (*4–6*), whereas other investigators have found a lower RBE for mutation induction than for cell inactivation after exposure to α particles of CHO cells (*7*). In a recent paper Thacker *et al.* (*8*) showed that V79-4 hamster cells respond to α particles from plutonium sources as was predicted by their previous results, having an RBE for cell killing lower by a factor of approximately two than that for mutation induction. Several possible technical and biological reasons for this difference in response have been discussed, but it was not possible to find a clear explanation.

[1] Present address: Radiobiology Laboratory, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44106.

0033-7587/84 $3.00

Since knowledge of the effectiveness of α particles for inducing mutations might be useful in radiation protection, it is important to examine the possible reasons for this variability. We have therefore studied the effects of α particles from ^{241}Am on cell inactivation and mutation induction using Ehrlich ascites tumor cells as biological system and have compared the results with those obtained after exposure to X rays.

MATERIAL AND METHODS

Ehrlich ascites tumor (EAT) cells grown in suspension in A2-medium as described previously were used (*9*). The mutation experiments were started with cultures containing about 500 cells in 2 ml of fresh medium which were allowed to grow in the presence of 4×10^4 feeder cells for 5–6 days. After this time the cells were passaged by dilution to 2×10^5 cells/ml once or twice and were then used to prepare cultures containing 10^5 cells/ml. Later, 3 days, the cultures entered the plateau phase of growth at a concentration of about 2×10^6 cells/ml; they were used for the experiments on Day 4.

Cells were irradiated under aerobic conditions either in suspension (2×10^6 cells/ml) with X rays (150 kV, 0.8 Al filter, $\dot{D}$ = 10 Gy/min) or on membrane filters with α particles from an ^{241}Am source. This had an active surface of 20×25 mm, an initial particle energy of E_0 = 5.49 MeV, a 1-μm gold foil cover, and an effective energy of emitted particles of E_{eff} = 4.69 MeV. The energy of the particles reaching the cell nucleus was calculated to be E_n = 3.8 ± 0.2 MeV. The dose rate at 1 mm was 2.2 Gy/min and the uniformity of the surface emission of the source was better than 10%. The energy spectrum of the source was measured using a detector calibrated against a standard source including ^{239}Pu (E = 5.15 MeV), ^{241}Am (E = 5.49 MeV), and ^{244}Cm (E = 5.82 MeV).

For irradiation with α particles, cells were layered on membrane filters (Millipore, 0.22-μm pore diameter, 13-mm filter diameter). For this purpose plateau-phase cells were concentrated to 10^7 cells/ml and 50 μl of suspension were gently spread on the filter, kept on a moist glass fiber pad soaked with cell-free medium from plateau phase culture. Exposure to the particles was carried out when the droplet was completely absorbed into the glass fiber pad leaving the cells as a monolayer on the surface of the filter. The extent of overlap of the cells was deduced to be very limited as indicated by the exponential form of the survival curve for exposure to α particles down to a survival fraction of 0.005. This assumption is further supported by the observation that decreasing the number of cells in the suspension, and therefore the number of cells on the filter, did not change the survival curve. After irradiation complete recovery of the cells from the filters was effected by gently shaking them in fresh medium. This procedure did not influence the plating efficiency or the radiosensitivity of the cells. Because a high number of cells was needed for mutation experiments, we irradiated 10 filters for each point and pooled the cells (about 5×10^6 cells total). After irradiation, a small proportion of the cells was plated at a low concentration (after addition of 4×10^4 feeder cells/ml exposed to 50 Gy X rays) on agar medium to measure the survival and the rest was incubated in 100 ml fresh medium and allowed to grow to effect expression of the induced mutants.

After 3 days the cells were at the end of the exponential growth and were diluted again in 100 ml fresh medium at 5×10^4 cells/ml (controls) or correspondingly more for the irradiated samples. After 6, 9, and 12 days this procedure was repeated and simultaneously cells were selected for induced resistance to 1.5 μg/ml 6-thioguanine (6-TG, added from a 10-mg/ml stock solution). For this purpose 5×10^6 to 10^7 cells were inoculated in 80 ml agar medium prepared by mixing one part of medium prepared at twice the normal concentration with one part of 1.1% agar (Difco). The number of viable cells was estimated by similar plating in 6-TG free medium after addition of 5×10^6 feeder cells (corresponding to 6×10^4 feeder cells/ml). Subsequently the cells were plated into three 10-cm petri dishes. The cells grew under these conditions well separated from each other, and problems due to "cross feeding" were not observed (see also Ref. *13*). The colonies were counted after 14 days' incubation at 37°C (6% CO_2). The plating efficiency was between 50 and 70%. More than 100 colonies were counted for each experimental point; the standard counting errors in the estimation of the survival and the mutant number counts were thus a maximum of 10%; the error in mutant frequency data was correspondingly less than 14%. A more detailed description of the methods used to select 6-TG^R mutants is presented elsewhere (*14*).

The X-ray dose was measured with an energy-independent ferrous sulfate dosemeter (*10*). The α-particle dose was measured with a parallel plate ionization chamber and also estimated from measurements based on the fluence rate. The α-particle dose values obtained by applying both methods were very similar and

agreed with those calculated using a semi-empirical formula of logarithmic dependence of the dose rate from the distance. These methods will be more extensively discussed elsewhere (Bertsche, manuscript in preparation). The LET of the particles leaving the source was $L = 100 \pm 10$ keV/μm, but when the air gap between the source and the cells monolayer as well as the thickness of the cells was considered, the LET at the center of the cell was calculated to be $L = 125 \pm 12$ keV/μm.

RESULTS

In the left-hand panel of Fig. 1 are shown the survival curves obtained when cells derived from the same culture were irradiated either in suspension with X rays or as a monolayer on membrane filters with α particles. Open and closed symbols represent two independent experiments. The D_0 value of the exponential part of the X-ray survival curve is 0.8 Gy, whereas the D_0 value of the α-particle survival curve was estimated by regression analysis to be 0.6 Gy. Presumably due to variations in the growth conditions the X-ray survival curve had a reduced shoulder width (about 1 Gy) compared to that of 2.2 Gy measured in previous experiments represented by the interrupted line in the figure. This effect was repeatedly observed in EAT cells when the batch of horse serum used was changed, and has been also discussed in previous publications (*12*). Recloning of the cultures or production of cultures starting

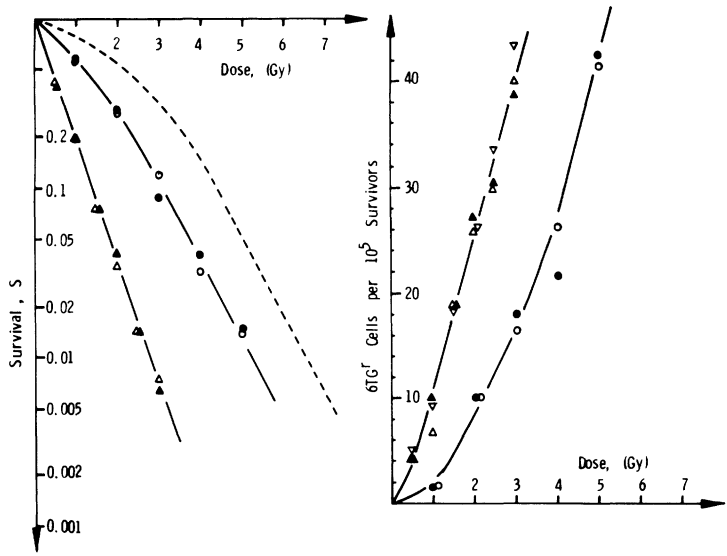

FIG. 1. Cell survival (left-hand panel) and induction of resistance to 6-TG (1.5 μg/ml) (right-hand panel) in plateau-phase (4-day-old) cells as a function of the absorbed dose, plated and processed for the measurement of induced mutants and survival immediately after irradiation. The triangles represent results obtained after exposure to α particles, circles results after exposure to X rays. Open and closed symbols represent different experiments. The same cell suspension was used for X and α-particle irradiations. The dotted line in the left panel is the survival curve obtained in previous experiments after exposure to X rays (see text). The number of mutants induced per 10^5 viable cells was measured after 9 days expression time. The inverse open triangles represent results obtained after 12 days expression time. The curves were fitted to the points by eye, except for the induction of mutations after α-particle exposure where linear regression analysis was applied to fit the point on a straight line.

from very low cell numbers, as applied for mutation experiments, did not affect this behavior. We have found indications that the shoulder width of the survival curve might arise from repair of PLD (*11, 12*) and assume therefore that the variations observed in cell survival reflect different abilities of the cells to repair PLD. The possible implications of this observation will be discussed in the next section. It is worth noting that this modification of cell survival was limited to the X-ray survival curve; the α-particle survival curve was not measurably influenced as indicated by comparison with previous results. This is not surprising since it is known that the amount of repair observed at the cellular level after exposure to sparsely ionizing radiation is much larger than that observed after exposure to density ionizing radiations (*13*). A change in the repair capability of the cells therefore is expected to affect mainly the X-ray survival curve. The RBE values calculated at survival values of 0.7, 0.37, and 0.1 are 3.25, 2.65, and 2.3, and when the previously obtained survival curves (dashed line) are considered, 6.25, 4.5, and 3.1, respectively.

In the right-hand panel of Fig. 1 are shown the results for the induction of 6-TG resistance after exposure to either X rays or α particles in the same dose range as that in which the cell survival was measured. The background of spontaneously arising mutants ($1–4 \times 10^{-5}$) was subtracted before plotting the data. The expression time was 9 days except for the data shown by the inverse triangles which were obtained after 12 days expression time. Since no significant change in the number of mutants induced was observed between 9 and 12 days expression time, we assume that at 9 days the maximum number of induced mutants was measured. Shorter expression times did not give maximal numbers of mutants, especially at high doses. Both curves reach a linear induction rate after a "shoulder region." Linear regression analysis for the α-particle results gave a slope of 14.1×10^{-5} Gy^{-1}. The dose intercept for the α-particle line is 0.2 Gy, whereas the linear part of the X-ray curve apparently reached at high doses can be extrapolated back to a dose intercept of 2.2 Gy. The small shoulder observed for the α-particle mutation induction curve was not observed in all experiments performed (four in total) and might be due to the standard error ($\sim$14%) in the estimation of the background of spontaneously arising mutants. The results shown for the induction of mutations after exposure to X rays differ only slightly from those previously measured (*14*).

It is evident from the results in Fig. 1 that α particles are more effective in inactivating cells and in inducing 6-TG resistant mutants when the comparison is made at the same dose. This higher effectiveness of α particles disappears when the comparison is made at the same survival level as indicated in Fig. 2. A straight line with a slope of 20×10^{-5} can be drawn through the experimental points representing exposure to either X rays or α particles. This fact is also indicated by the RBE values obtained at mutation induction levels of 1.2×10^{-5}, 5.4×10^{-5}, and 17.4×10^{-5} (corresponding to X-ray survival levels of 0.7, 0.37, and 0.1, respectively) which were 3.25, 2.46, and 2.14, and not significantly different from those for cell killing, which were 3.25, 2.65, and 2.30 at the above survival levels.

DISCUSSION

The results presented in the previous section indicate a more effective induction of mutations by α particles than by X rays, as was found for cell killing, when the

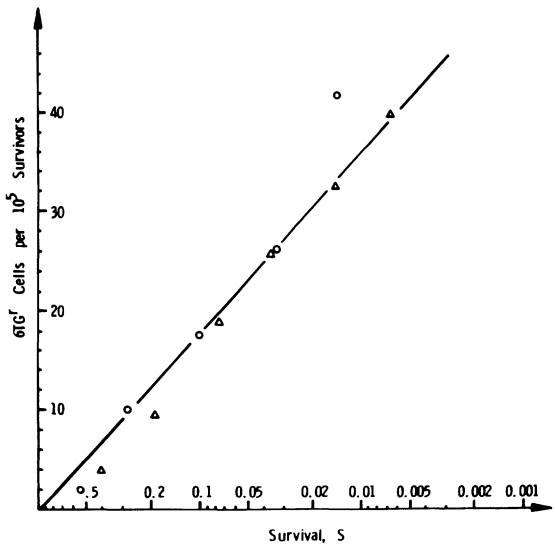

FIG. 2. Induction of 6-TG resistance in plateau-phase cells as a function of cell survival for cells exposed to X rays (circles) or to α particles (triangles). Values derived from Fig. 1.

comparison is made at the same dose level. The RBE values measured for the two end points were very similar. This result contrasts the finding of Thacker *et al.* (*8*) where a higher RBE was observed for mutation induction than for cell inactivation and also differs from the results of Barnhart and Cox where a lower RBE was observed for induction of mutations than for cell inactivation. These differences are also reflected in the plot of induced mutants as a function of survival shown in Fig. 2. The same straight line could be drawn through the points after exposure to both X rays and α particles. The line thus obtained is practically identical to that measured by Thacker *et al.* (*8*) for α particles, indicating that the difference from their results is mainly due to a higher induction rate of mutants per survivor in Ehrlich ascites tumor cells after exposure to X rays. Since the influence of repair processes on cell survival is more pronounced after exposure of cells to low than to high-LET radiations (e.g., Ref. *13*), we suggest that the discrepancy observed after exposure to low-LET radiations might reflect differences in the ability of the cells to deal with reparable lesions resulting in either mutation or cell inactivation. EAT cells probably deal under our experimental conditions with reparable mutagenic lesions less effectively than with potentially inactivating lesions, thus producing a higher number of mutants for a certain survival level than V79-4 Chinese hamster cells.

The difference between our results and those of Barnhard and Cox is mainly due to the low induction of mutants observed by these authors in CHO cells after exposure to α particles. The induction curve of mutants as a function of the dose of α particles was almost linear for EAT cells and V79-4 Chinese hamster cells but upward bending for CHO cells. The lower efficiency of induction of mutants per survivor in the case of CHO cells is nevertheless restricted to the low-dose region; when a comparison is made at high doses the values obtained are comparable to those obtained with EAT

and V79-5 cells, indicating the induction of 30 mutants per 10^5 cells at a survival level of about 1%. This is comparable to the value of 35 found for EAT cells at the same survival level or in V79-4 cells under similar conditions.

The lower rate of induction of mutants coupled with a normal radiosensitivity of CHO cells after exposure to low doses of α particles (up to about 2.5 Gy) could be peculiarity of the cell line but could also be the result of loss of mutants in the low-dose region under the culture conditions and at the expression times used. If the former explanation holds, the difference in the results would indicate that lethal and mutagenic lesions are induced or treated by the repair system after exposure to α particles in a way which is different for the two kinds of lesions varying from cell line to cell line and are therefore described by different mathematical functions. The present results do not allow a final conclusion on this, but experiments with EAT cells have indicated that potentially mutagenic and potentially lethal lesions are affected by postirradiation treatments of the cells in a similar way (*14*).

CONCLUSION

A higher number of 6-TG resistant cells was found to be induced after exposure of EAT cells to a given dose of α particles than to X rays. The RBE for this induction was similar to that for cell inactivation, contrary to the results obtained with other cell systems where either a higher or lower RBE was observed for the induction of mutations than for cell inactivation. The reason for this variability between the various cell lines could be (except possible cell line peculiarities or methodical errors) a different ability of the cells to deal with reparable mutagenic and potentially inactivating lesions, which are predominantly induced after exposure of cells to low-LET radiations.

RECEIVED: June 10, 1983; REVISED: December 15, 1983

REFERENCES

1. G. W. BARENDSEN, Impairment of the proliferative capacity of human cells in culture by α-particles with differing linear-energy transfer. *Int. J. Radiat. Biol.* **8,** 453–466 (1964).
2. P. W. TODD, *Reversible and Irreversible Effects of Density Ionizing Radiations upon the Reproductive Capacity of Cultured Human Cells,* pp. 2–14. Medical College of Virginia, 1966.
3. D. T. GOODHEAD, R. J. MUNSON, J. THACKER, and R. COX, Mutation and inactivation of cultured mammalian cells exposed to beams of accelerated heavy ions. IV. Biophysical interpretation. *Int. J. Radiat. Biol.* **37,** 135–167 (1980).
4. R. COX, J. THACKER, D. T. GOODHEAD, and R. J. MUNSON, Mutation and inactivation of mammalian cells by various ionizing radiations. *Nature (London)* **267,** 425–427 (1977).
5. J. THACKER, A. STRETCH, and M. A. STEPHENS, Mutation and inactivation of cultured mammalian cells exposed to beams of accelerated heavy ions. II. Chinese hamster V79 cells. *Int. J. Radiat. Biol.* **36,** 137–148 (1979).
6. R. COX and W. K. MASSON, Mutation and inactivation of cultured mammalian cells exposed to beams of accelerated heavy ions. III. Human diploid fibroblasts. *Int. J. Radiat. Biol.* **36,** 149–160 (1979).
7. B. J. BARNHART and S. H. COX, Mutagenicity and cytotoxicity of 4.4-MeV alpha particles emitted by Plutonium-238. *Radiat. Res.* **80,** 542–548 (1979).
8. J. THACKER, A. STRETCH, and D. T. GOODHEAD, The mutagenicity of alpha particles from Plutonium-238. *Radiat. Res.* **92,** 343–352 (1982).
9. G. ILIAKIS and W. POHLIT, Quantitative aspects of the repair of potentially lethal damage in mammalian cells. *Int. J. Radiat. Biol.* **36,** 649–658 (1979).

10. D. FRANKENBERG, A ferrous sulfate dosemeter independent of photon energy in the range from 25 keV up to 50 MeV. *Phys. Med. Biol.* **14,** 597–605 (1969).

11. G. ILIAKIS, Effects of β-arabinofuranosyladenine on the growth and repair of potentially lethal damage in Ehrlich ascites tumor cells. *Radiat. Res.* **83,** 537–552 (1980).

12. G. ILIAKIS, Characterization and properties of repair of potentially lethal damage as measured with the help of beta-arabinofuranosyladenine in plateau-phase EAT cells. *Radiat. Res.* **86,** 77–90 (1981).

13. V. BERTSCHE and G. ILIAKIS, Radiation quality and repair of potentially lethal damage. In *Eighth Symposium on Microdosimetry,* Julich (J. Booz and H. G. Ebert, Eds.), pp. 383–395. Commission of the European Communities, Luxembourg, 1982.

14. G. ILIAKIS, The influence of conditions affecting repair and fixation of potentially lethal damage on the induction of 6-thioguanine resistance after exposure of mammalian cells to X-rays. *Mutation Res.,* in press.