RESPONSE OF MOUSE EPIDERMAL CELLS TO SINGLE DOSES OF HEAVY-PARTICLES

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The survival of mouse epidermal cells to heavy-particles has been studied In Vivo by the Withers clone technique. Experiments with accelerated helium, lithium and carbon ions were performed. The survival curve for the helium ion irradiations used a modified Bragg curve method with a maximum tissue penetration of 465 microns, and indicated that the dose needed to reduce the original cell number to 1 surviving cell/cm^2 was 1525 rads with a D_o of 95 rads. The LET at the basal cell layer was 28.6 keV/micron. Preliminary experiments with lithium and carbon used treatment doses of 1250 rads with LET's at the surface of the skin of 56 and 193 keV/micron, respectively. Penetration depths in skin were 350 and 530 microns for the carbon and lithium ions whose Bragg curves were unmodified. Results indicate a maximum RBE for skin of about 2 using the skin cloning technique. An attempt has been made to relate the epidermal cell survival curve to mortality of the whole animal for helium ions.

The cell survival curves for single doses of 29 kVp x-rays, 250 kV x-rays and fast neutrons have already been defined (refs. 1 to 3) for mouse skin epithelial cells. It was felt that survival of mouse skin epithelial cells after heavy-particle irradiation would be of interest. This consideration is particularly relevant for the space environment where an astronaut might conceivably meet particles of high LET and relatively low penetration power. Indeed, the greatest radiation hazard to astronauts is exposure to particle events, with alpha particles making up a considerable fraction of the total particle flux (refs. 4 to 6). In this regard, the In Vivo epidermal cell cloning technique of Withers (ref. 1) has been used.

METHOD

A circular treatment area (25 mm diameter) on the animals ventral surface was irradiated. In this area small aluminum shields (20 mil thickness) of varying diameters were placed. Shields of 19.0, 6.4, and 2.1 mm diameter were used, and respectively, 1, 3, or 7 shields were placed within the 25 mm diameter irradiation field. Initially, the circular area was irradiated without the shields. Then, the shields were mechanically pulled into place and a large dose (i.e. 10 krad) was given to insure that cells from the periphery would not regrow into the treated areas. At intervals post-irradiation, the areas were examined for epidermal cell regrowths which could be seen as visible whitish clones. Assuming that each clone arises from a single surviving epidermal cell, the curve of cell survival/cm^2 may be obtained. If in a number of similarly treated areas the average number of surviving cells is m, the probability that 0, 1, 2, 3, etc., cells will survive will follow

a Poisson distribution with mean of m. If, in a number of equal areas (A), a fraction (f) shows regrowth after irradiation, the number of surviving clonogenic cells per unit area (S) is:

$$S = - \ln (1 - f) / A$$

By varying A, the test area, about 3 decades of a cell survival curve may be obtained.

Male, hairless mice of the CD1 strain were obtained from the Charles River Breeding Laboratories, Cambridge, Massachusetts. Mice were between 10-14 weeks of age at irradiation, and were singly housed. The animals were anesthetized for irradiation by intraperitoneal injections of Nembutal (60 mg/Kg body weight).

The helium ion exposures were performed at the Lawrence Radiation Laboratory 88'' cyclotron. Animals were individually irradiated and were positioned in a vertical manner normal to the direction of the incident helium ion beam. A large graphite shield was used so that only the circular area on the ventral surface of the animal was exposed. Photographic and miniature semiconductor diode measurements indicated that the beam intensity was homogeneous over the area irradiated. The helium ion beam had an initial energy of 40 MeV which is equivalent to a range of 90 mg/cm^2 in mylar (approximately 8% less in tissue). Mylar absorbers were placed in the beam path to produce a maximum residual range of 465 microns tissue (about 51 mg/cm^2 mylar). The single Bragg curve was modified into a family of Bragg curves occurring at different depths in tissue by placing a series of very thin overlapping mylar absorbers into the beam path. The absorbers rotated at 200 RPM through the beam to produce variable absorption with production of many Bragg curves which gave a flattened depth dose distribution. The "average" Bragg curve is shown in Figure 1. The average curve rose from a relative ionization factor of 1.1 at the surface of the skin to 2.1 at its maximum value at about 400 microns penetration. Doses were expressed at the top of the skin, and the minimum LET at this point was 28.6 keV/micron.

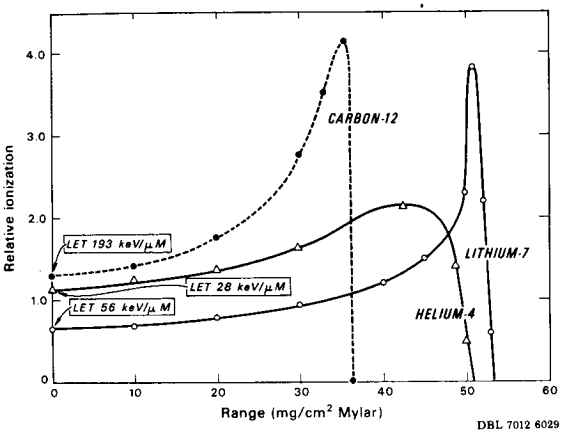

Figure 1. Depth dose curves for helium, lithium and carbon ions. The helium ion depth dose curve is formed by superimposition of separate Bragg curves which are produced by rotating varying layers of mylar absorber through the beam path with production of a resultant "average" Bragg curve which is relatively flat over its range with a well defined maximum penetration depth. Dose expressed at the top of the skin. The lithium and carbon ion curves are unmodified.

The lithium and carbon ion experiments were performed at the Lawrence Radiation Laboratory Heavy-Ion Linear Accelerator (HILAC). Initial energies of both beams were approximately 10 MeV/AMU. The lithium ion beam was degraded further in energy by interposition of mylar absorbers in the beam path. The carbon ion beam was not additionally degraded. The Bragg curves and their maximum penetration depths in mylar are also shown in Figure 1. Doses were expressed at the surface of the skin and the Bragg curves were unmodified. Doses of 1250 rads were given, with three treatment areas per mouse. Doses were measured with an integrating ionization chamber which interrupted the beam automatically after a preset dose. Average dose rate was 5000 rads per minute.

The scoring of all the epithelial survival responses was done by one observer. As pointed out by Emery et al (ref. 2), the response is quite subjective. Mice were observed from day 7 post-irradiation onward until the response could be considered complete. Ulceration of the skin appeared at different times post-irradiation with the order of appearance being carbon, lithium, and helium ions. The ulcerations appeared on about days 10, 13, and 14, respectively. Clones were observable from about day 10 onward to a maximum of about day 20. The clones differed in appearance; some being punctate, while others were more diffuse and appeared as thin sheets. Only clones which were scoreable for two or more observation periods in a row were considered positive identifications (i.e. about 4-7 days). Histological samples were taken for further identification. Due to stretching of the skin of the anesthetized animal during irradiation, the irradiation fields were often not circular but elongate. There were also scoring difficulties when extensive scab formation was seen. If there was any question as to the patency of the irradiation procedure the animal in question was excluded from the analysis. Still, as pointed out by Emery et al (ref. 2), these possible uncertainties in scoring should not shift experimental survival points by a great deal. Experiments were not subject to errors induced by fortuitous clonal regrowth via hair follicles.

RESULTS

Table I lists the surviving cells/cm^2 for each of the ions and doses.

Figure 2 shows the results obtained after irradiation for the helium ion exposures. The points fit a linear relationship of log survival to linear helium ion dose over the range of doses studied. A regression analysis of the unweighted points yields a D_o value of 95 rads, and extrapolation of the curve to zero dose produces a surviving cell number/cm^2 of 1.5×10^8. Epithelial survival curves for 29 kVp x-rays (ref. 1) and 250 kV x-rays (ref. 2) have been included for comparison purposes.

Included also in Figure 2 are the survival values/cm^2 for the single 1250 rad doses for the lithium and carbon ion irradiated animals. The points lie to the left of the helium ion or x-ray dose response curves.

TABLE I

Epithelial Cell Survival Results For Single Heavy-Particle Exposures

No. of Experimental Areas per mouse	Area (cm^2)	Dose	No. of Areas Irradiated	No. of Areas Regrowing	Average Survival Cells/cm^2
Helium Ions					
1	2.85	1625	30	20	0.251 (.21-.30)*
1	2.85	1750	30	8	0.185 (.15-.20)
3	0.317	1250	45	34	2.45 (2.14-2.76)
3	0.317	1500	84	15	1.41 (1.28-1.54)
3	0.317	1750	72	5	1.27 (1.19-1.35)
7	0.035	1250	35	12	15.1 (12.6-17.6)
7	0.035	1375	35	7	13.3 (11.4-15.3)
7	0.035	1500	35	2	11.8 (10.6-12.7)
Lithium Ions					
3	0.317	1250	78	20	1.50 (1.36-1.59)
Carbon Ions					
3	0.317	1250	75	13	1.40 (1.27-1.54)

*Numbers in parentheses indicate 95% confidence limits

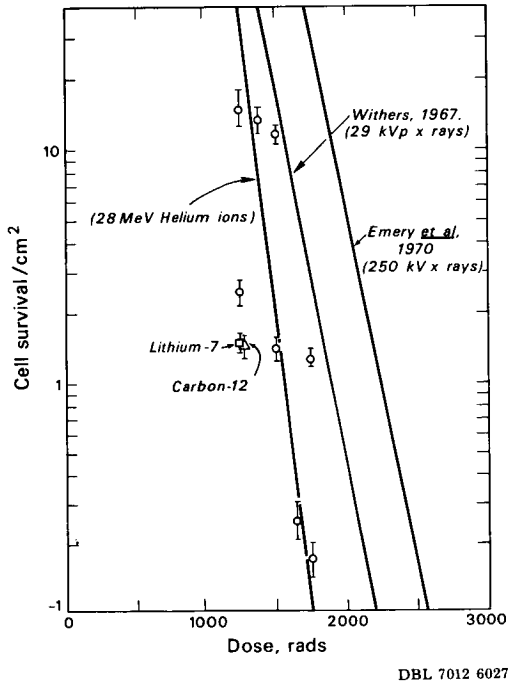

Figure 2. Survival of Epithelial cells following single doses of heavy particles. Vertical bars indicate 95% confidence limits.

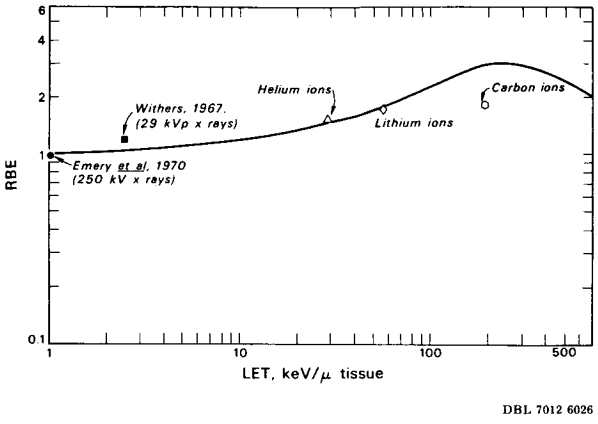

Figure 3. Schematic curve showing variation of RBE with LET from experimental data. The curve shown in for mammalian cells (ref. 7).

DISCUSSION

Results have been interpreted in terms of the estimated number of surviving cells/cm^2, assuming that each visible clone has regrown from a single cell. The heavy ions appear to have a greater effect than either 29 kVp x-rays (ref. 1) or 250 kV x-rays (ref. 2). Indeed, if one examines the doses needed to reduce epithelial cell survival to a level of 1 surviving cell/cm^2, one can obtain a tentative RBE versus LET response (Figure 3). The curve for mammalian cells has been derived from heavy ion experiments on cells in culture (ref. 7). The RBE values for x-rays (ref. 1 and 2) and the heavy ions are listed in Table II, together with other experimental values. The 250 kV x-ray work of Emery et al (ref. 2) has been used as the comparison work. Tentative doses for lithium and carbon ions have been found assuming that the cell survival/cm^2 intercept at zero dose will be the same as that found with helium ion exposure and drawing a line from this point through the observed cell survival/cm^2 extending to 1 surviving cell/cm^2 As extrapolation numbers decrease with increasing LET, the assumption that zero dose cell survival values will be equivalent for the helium, lithium, and carbon ions may be incorrect. However, the possible error from this in estimation of the 1 cell/cm^2 survival level should not be very large. The RBE-LET response curve lies below the schematized curve for mammalian cells. The data suggest that the RBE-LET response for skin reaches a plateau of only about 2 for heavy ions as compared to the value of about 3 for other mammalian systems In Vitro. Further support for a maximum RBE value of about 2 is presented by Denekamp et al (ref. 3) who show a maximum RBE of about 2

TABLE II

Researcher	Strain of Mouse	Do Rads	Y Intercept	Radiation Quality	RBE	LET_∞^{II} (keV/uM)
Emery et al (2)	Male Albino SAS/TO, 8-9 weeks of age at exposure	135	1.35×10^7	250 kV x-rays (300 rads*/ minute) III	1.0^I	1.0
Withers (1)	Inbred Albino WH/Ht, both sexes, 9-14 weeks of age at exposure	135	1.39×10^6	29 kVp x-rays (769 rads/ minute)	1.2	3.0
Leith et al	Male CD1 Hairless, random bred, 10-14 weeks of age at exposure	95	1.50×10^8	^{4}Helium (3000 rads/ minute)	1.6	28.0
				^{7}Lithium (5000 rads/ minute)	1.9	56.3
				^{12}Carbon (5000 rads/ minute)	2.0	192.8

*dose rates of treatment exposures

(All mice were anesthetized at the time of exposure)

[I] RBE values taken as the doses needed to reduce cell survival/cm^2 to 1.0 using the data of Emery et al (2) as basis of comparison.

[II] LET_∞ denotes the total particle linear energy transfer including all secondary electrons.

[III] 250 kV irradiation HVL = 1.5 mm Cu

with fast neutron (mean energy 8 MeV) using the same experimental system. Expressing survival curve responses as a function of radiation dose at the epidermal basal cell layer is probably the correct way. The basal layer in our hairless CD1 mouse lies approximately 20-30 microns below the top of the ventral skin. If radiation doses were expressed as the average dose across the radiation penetration range, or as the maximum dose, which would be found close to the maximum penetration depth, the observed cell survival figures for the helium, lithium, and carbon ions would be shifted to the right (see Figure 2). Such a result does not seem probable, as one would expect some degree of greater efficiency of cell killing with higher LET particles.

RELATION OF CELL SURVIVAL TO WHOLE ANIMAL MORTALITY

Radiation of the skin of the mouse with helium ions kills a proportion of the basal cell layer of the skin. If enough of these cells are killed, this should be critical to the survival of the organism, and its fate will depend on the remaining number of surviving cells. It is of great importance to relate such whole animal mortality to the survival curves for individual cells as has been attempted for other cell systems (refs. 8 to 10). Such data is available for whole animal mortality after helium ion irradiation of the skin. In this approach a slightly different technique has been used where the animal rotates in front of the helium ion beam while enclosed in mylar holders. As the animal rotates each point on the skin will see a spectrum of Bragg curves from maximum penetration (set at 500 microns tissue) to zero penetration. Such a spectrum of curves also produces a flattened depth dose distribution very similar to Figure 1 for the helium ions. Irradiation in this fashion produces a skin damage syndrome which may be lethal to the animal. Animals that die, do so within 22-42 days post-irradiation with a mean survival time of about 29 days. The mortality response is dose-dependent, and shows an LD50/60 of 1543 rads (expressed as the dose at the top of the skin). An approximate 0.1% threshold of 1080 rads may be found from extension of the mortality response versus dose to the 0.1% mortality level. A value of 500 rads may be given for K, where K is the mortality curve probit width; i.e. the dose which causes a unit change in the probit of mortality.

If one considers the mortality response to be primarily a function of the number of surviving epidermal cells, the LD50 corresponds to about 2 surviving cells/cm^2,

Lange (ref. 11) from analysis of the Withers data (ref. 1) lists the extrapolation value for the mouse epidermal cell survival curve/cm^2 to be about 6.5. Gilbert(ref. 12) gives an equation for the relation of cell survival to whole animal mortality of:

$$\text{probit } (Pm) = (D - D_o \cdot \ln (NaE)/(D_o / B)$$

Where $LD_{50} = D_o \cdot \ln (NaE)$

and $K = D_o/B$.

As stated by Gilbert (ref. 12), the LD_{50} does not depend on the sensitivity factor (B), but is just the dose needed to reduce the sensitive cell number to a critical level. The factor Na is simply the ratio of the normal sensitive cell number to the critical number. The probit width K is not dependent on N, a, or E and is proportional to D_o.

As illustration, for the whole animal helium ion irradiations at a depth of 500 microns, the LD50 is 1543 rads, and K is 500 rads. For the epidermal cell survival curve at an irradiation depth of 500 microns, D_o is 95 rads, and E is 6.5 (ref. 11). Using the above equations, Na becomes 1.74×10^6 and B is 0.19. If one assumes that there are about 1×10^6 epidermal cells/cm^2 and that the mouse has a total surface area of about 40 cm^2, then N, the normal stem cell level is 40×10^6 cells and a then equals 4.35×10^{-2}. As comparison, the value of a for the bone marrow radiation syndrome is about 2.3×10^{-3} (ref. 12). Gilbert lists a critical level where there is 50 per cent survival of S = 1/a. For mouse cells this becomes S = $1/4.35 \times 10^{-2}$ or about 2.3×10^1 cells per animal. Again, for an animal with a surface area of 40 cm^2 this is about 0.6 cells/cm^2 surviving at the whole animal 50% mortality level. From our results, we empirically show that at the LD50 dose (1543 rads) that this should give about 2 cells surviving/cm^2, a finding not too far at odds with that calculated using Gilbert's data (ref. 12). It is interesting that Withers (ref. 1) states "10-20 cells must be capable of preventing ulceration by proliferating to cover a 1 cm^2 area.....the response of skin to irradiation is therefore critically dependent on very few cells".

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