SURVIVAL OF V79 CHINESE HAMSTER CELLS IRRADIATED WITH HIGH-LET PROTONS: IMPLICATION FOR THE RBE-LET RELATIONSHIP.

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INTRODUCTION

Information about the biological efficiency of high linear energy transfer (LET) radiations is considered relevant to radiation protection because of:

(i) the increasing use of densely ionizing particles in several human activities;

(ii) the need for a better understanding of the basic mechanism of radiation action.

A particular interest should be deserved to the biological effects of low-energy protons. Neutrons exert their action mainly through proton recoils in the MeV range and a great part of the dose delivered by high-energy protons used for therapeutical applications is concentrated in the low-energy, high-LET region near the path end (Bragg peak). However, experimental work in this field is scarce and limited to relatively high energies.

We have studied the survival of V79 Chinese Hamster cells irradiated with proton beams in the energy range of 0.73-3.36 MeV, corresponding to an average LET inside the cells of 10.6-34.5 keV/μm.

MATERIALS AND METHODS

Irradiations with monoenergetic proton beams have been performed using the facility for radiobiological studies, set up at the 7 MV Van de Graaf accelerator of the INFN-Laboratori Nazionali di Legnaro, Padova(1). X-rays produced by an apparatus operating at 200 kV with a 0.2 mmCu filter have been used as reference.

Survival curves have been obtained in the dose range 0.5-6 Gy, at the dose rate of 1 Gy/min. 18 hrs before irradiation, $1x10^5$ V79 cells were seeded in each stainless steel Petridish especially designed to fit the beam geometry. The cells were grown at 37 °C as a monolayer attached to a mylar foil, having an area of 133 mm^2, placed at the bottom of the dishes. The cell monolayer was exposed to the beam through the mylar foil.

Immediately after irradiation the cells were detached by trypsin-EDTA treatment, counted, diluted and plated at the appropriate concentration. After 8 days of incubation at 37 °C, the survival was calculated by scoring the number of visible clones.

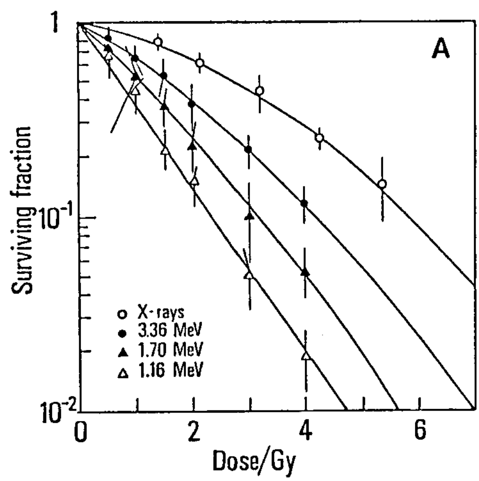
RESULTS AND DISCUSSION.

Fig.1 reports the survival curves for V79 cells irradiated in air with protons having energies of 3.36, 1.62, 1.16 MeV (Fig.1 A), 0.84 and 0.73 MeV (Fig.1 B), in the dose range 0.5-6.0 Gy. The energy values are referred to the incident surface of the cells. Both LETs and doses are expressed as the average values calculated at 3 µm depth, assuming a thickness of 6 µm for the cell monolayer. Each data point represents the mean of 4-10 individual experiments with one standard error of the mean.

In fig.1A experimental data were fitted using the expression:

$$S = \exp(-\alpha D-\beta D^2)$$

Increasing the LET, the initial shoulder of the survival curves tends to disappear. Linearity is reached at 23.9 keV/µm where the β coefficient results statistically irrelevant.



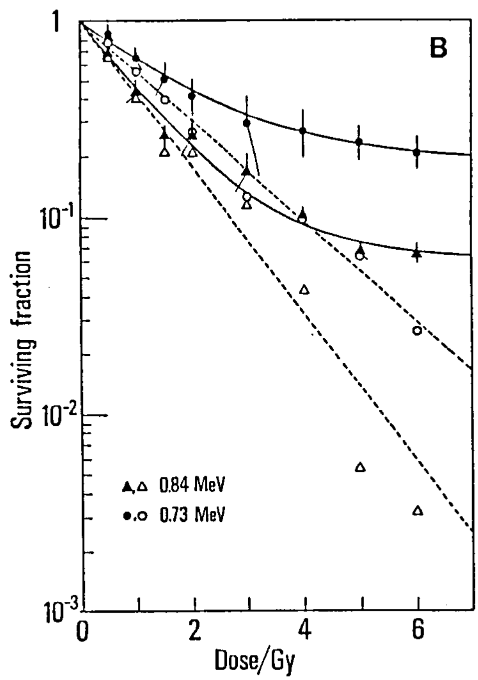


Fig.1 - Survival curves of V79 cells irradiated with proton beams in the energy range 0.73-3.36 MeV. Data obtained with X-rays are reported for comparison. Dotted lines in panel B represent the exponential parts of the fitting equation. The open symbols are the experimental data corrected for the fraction f.

For higher LET, such as those considered in Fig. 1B, the survival data show a tail at high doses, leading to apparent negative beta values when they are interpolated with the linear-quadratic expression. This effect cannot be explained in terms of LET variations and/or proton "loss" inside the cell monolayer. There are reasons to believe that it is presumably due to a portion of not flat, poorly attached or shielded cells receiving an attenuated dose of radiation. Assuming as a first approximation that a fraction f of cells has received no dose at all, we used the expression:

$$S = (1-f)\exp(-\alpha D)+f$$

to fit these data. The dotted lines show the fitting of the corrected data using the exponential part of the latter expression.

Table I lists the survival parameters obtained from the best fit procedures. The ratio α/α_x reported in the last column can be considered as the RBE at low doses. It can be seen that it increases with the LET, showing a maximum at about 24 keV/µm and decreases at higher LET values. Our data are consistent with those recently reported for 5.8 and 12.1 keV/µm protons (5).

Table I

Radiation	α/Gy^{-1}	β/Gy^{-2}	f	α/α_x
X-rays	0.128+/-0.023	0.046+/-0.005		1.0
Protons				
10.6 keV/µm	0.378+/-0.031	0.040+/-0.009		2.9
17.8 keV/µm	0.586+/-0.052	0.037+/-0.016		4.6
23.9 keV/µm	0.938+/-0.019			7.3
30.4 keV/µm	0.803+/-0.059		0.061+/-0.007	6.3
34.5 keV/µm	0.536+/-0.040		0.189+/-0.015	4.2

Interesting suggestions can be drawn comparing the RBE-LET relationships for protons and other particles (Fig.2). The curve appears shifted to lower LET values when compared to the results obtained with alpha particles and heavier ions (2, 3, 4). Therefore, in the rising part of the curve the RBE for protons is higher than that of alpha particles of comparable LET.

Preliminary data on the induction of $HGPRT^-$ mutants are consistent with this finding.

These results suggest that: (i) the LET could not be a suitable parameter of radiation quality and (ii) the quality factor evaluation based on the "classical" RBE-LET relationship (4) may not be of general validity. It appears that differences in the track structure, at the sub-micrometer level, among different types of radiation are of biological significance.

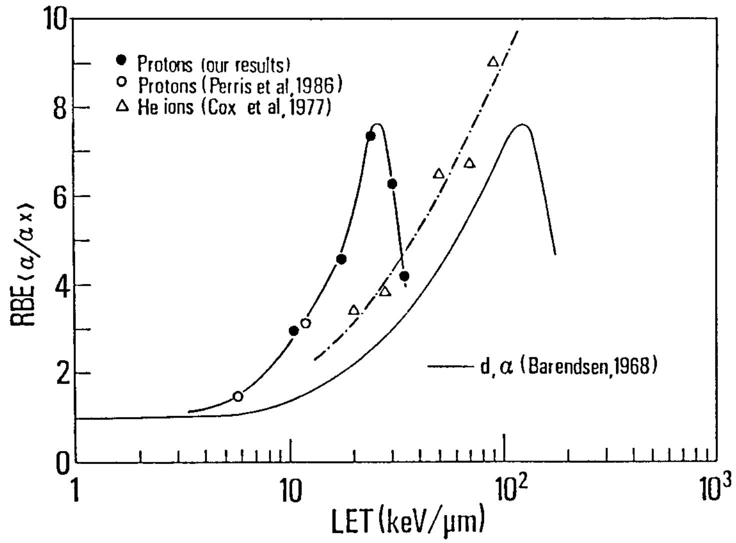


Fig.2 - RBE-LET relationships for protons and other particles.

This points out the importance of an approach based on the microscopic distribution of the energy deposited by radiation in relevant targets.

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