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Assessment of Acute and Late Effects to High-LET Radiation

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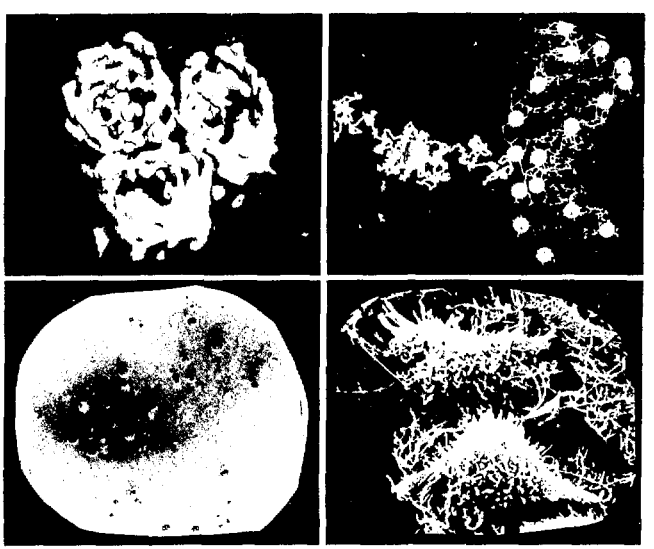

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DISCLAIMER

ASSESSMENT OF ACUTE AND LATE EFFECTS TO HIGH-LET RADIATION

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We have begun to reassess late tissue effects available from the Charged Particle Cancer Radiotherapy program at Berkeley. Our quantitative approach is limited in the analysis of these Phase I/II studies by not having equivalent patient numbers for each of the particle beams studied, by not having completely comparable follow-up times, by variations in the sizes of the fields compared, by variations in the skin scoring photographic documentation available from the patient charts, and by variations in the fractionation sizes, numbers and schedules. Despite these limitations, preliminary evidence demonstrates acute skin reactions with a shift to increasing lower dose per fraction per field for the maximum skin reactions of helium, carbon and neon ions compared to electrons. Comparisons with skin reactions from low-energy neutrons indicate that Bragg peak carbon ions (initial energy 308 MeV/nucleon) are slightly less effective than 7.5 MeV neutrons. Bragg peak neon ions (initial energy 670 MeV/nucleon) corrected for differences in reference radiation are slightly more effective than 7.5 MeV neutrons. Bragg peak silicon ions (initial energy 670 MeV/nucleon) result in an enhanced acute skin reaction, and a premature appearance of late effects that may indicate a significantly different mechanism of damage and/or repair.

INTRODUCTION

Little clinical information is available on the assessment of acute and late effects of normal tissues to high-LET charged particles. With the closure of the clinical treatment program at the Berkeley Bevalac Facility in 1992, the U.S. National Cancer Institute has supported a follow-up of the approximately 1300 patients who received charged particle radiotherapy for cancer at the Lawrence Berkeley Laboratory during the period from 1975-1992. A systematic assessment of normal tissue reactions appearing early and later after exposure to high energy helium (225 Mev/u), carbon (308 MeV/u), neon (670 Mev/u) and silicon (670 MeV/u) ions is a part of this program. This is a report of some preliminary information obtained from this study.

The development of tissue damage after irradiation is known to depend on a number of factors, including whether or not the tissue is proliferating or consists of non-dividing or terminally differentiating tissues. Either type of tissue generally demonstrates a three phase response (early, intermediate and late) that is dependent on the dose and linear energy transfer (LET) of the radiation. (1) With exposure to radiations having LET values of about 100 keV/μm, the time course and severity of the reaction can shift. Frequently there is the appearance of earlier acute and late effects in both proliferative and non-proliferative tissue. The early induction period frequently is short and the rise to the maximum value is faster; the level of intermediate damage is higher, and the

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time of appearance of late effects is earlier and the rate of any subsequent degeneration is faster (1).

Leith *et al*, (2) compiled a summary of normal tissue relative biological effectiveness (RBE) data from charged particle studies with experimental animals. At the same dose-averaged LET value, he reported a hierarchy of tissue responses, with for example, spinal cord having a much higher RBE value compared to intestinal crypt or skin. This was similar to observations made with neutrons by Field (3). It is important for clinical considerations to analyze RBE data from fractionated dose regimens.

Raju and Carpenter (4) evaluated the correlation between acute and late reactions to most of the available particle beams with a mouse foot skin model using plateau and Bragg Peak ions. They did not see any lack of correlation between the two. Acute effects were a good predictor of late effects. Both increased at high dose, but only single dose effects were measured.

This preliminary report summarizes PhaseI/II acute skin reaction studies that were completed for the purpose of selection of appropriate dose-fractions with charged particle beams at energies and LET values used for the first time for radiotherapy with the intent to eradicate underlying tumor. In the case of helium and silicon ions, correlative biological studies are also described. The analysis of late effects is still underway.

METHODS AND MATERIALS

Design of the study

We have deliberately selected the skin scores from patients, where due to the superficial location of their lesions, skin was not spared and received particle doses in the Bragg peak of the ions studied. The data therefore represent RBE estimates for normal tissues that could not be eliminated from the treatment volume.

Eligibility criteria

Only Phase I/II patients with locally advanced or metastatic disease who consented to the feasibility testing of charged particle exposures were included in the study.

Skin scoring

We have used an expanded 9-point scale (Table 1) to allow us to distinguish qualitative differences in erythema, and dry and moist desquamation. Electrons were used as a low-LET reference.

Table 1. CLINICAL SCORING FOR ACUTE SKIN IRRADIATION

1. No visible reaction
2. Minimal erythema
3. Moderate erythema
4. Minimal dry desquamation
5. Moderate dry desquamation (less than 1/2 field)
6. Marked dry desquamation (more than 1/2 field)
7. Moist desquamation (less than 1/2/ field)
8. Moist desquamation (more than 1/2 field)
9. Necrosis and/or ulceration

In vitro cell line and culture conditions

Asynchronously growing human T-1 cell fibroblasts (5,6) were used in the *in vitro* studies. There is a large data base in our laboratory with this cell line characterizing scattered, wobbled and scanned particle beams covering a range of atomic numbers from protons to uranium.

Particle exposure conditions

Cells were irradiated either in monolayers in tissue culture flasks filled with growth medium or in suspension under the conditions to be described later which simulated the patient irradiations under study. Dose-survival responses were obtained from single or sequential doses of the specified radiations.

In vitro survival measurement

A conventional colony-forming assay was used. After exposure the cells were trypsinized, resuspended, counted, plated, and incubated at 37°C for 11 days. Colony-forming was scored by staining the cultures with 1% methylene blue, and clones containing at least 50 cells were scored as survivors.

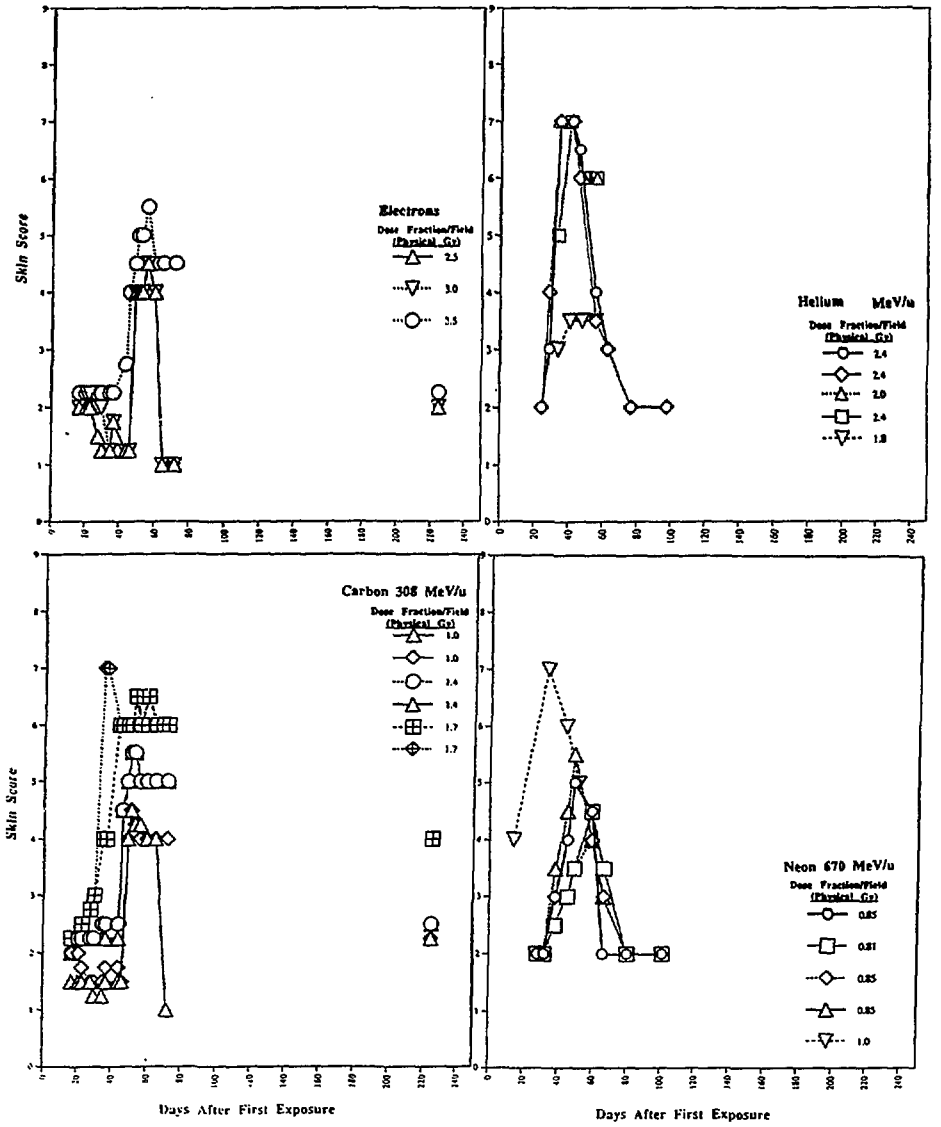

Fig. 1. Time course of maximum human skin responses to various accumulated dose fractions per treatment field of electrons, helium, carbon or neon ions. Note that data are from different dose fraction sizes and approximately equal fraction number, therefore total doses are not equivalent.

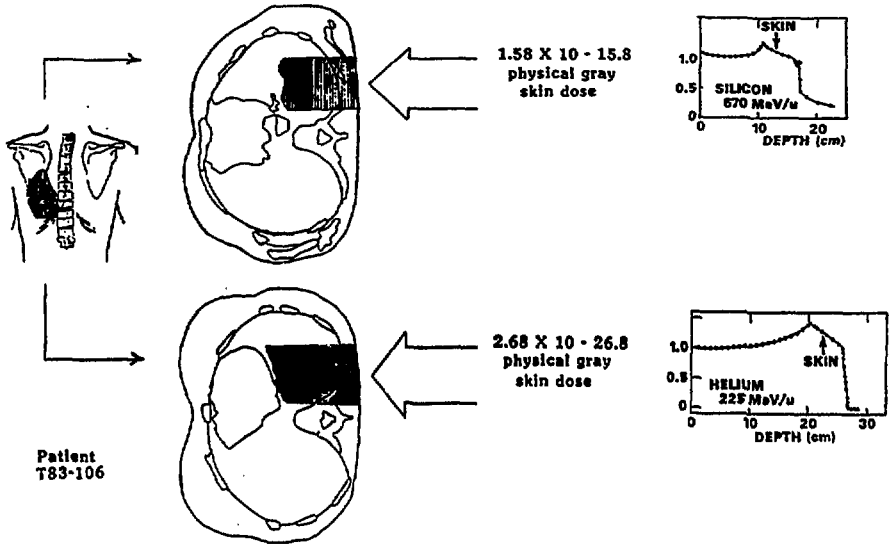

Fig. 2. Diagrammatic representation of two skin fields irradiated with either 225 MeV/u helium ions or 670 MeV/u silicon ions in a patient being treated for lung nodules with two matching fields.

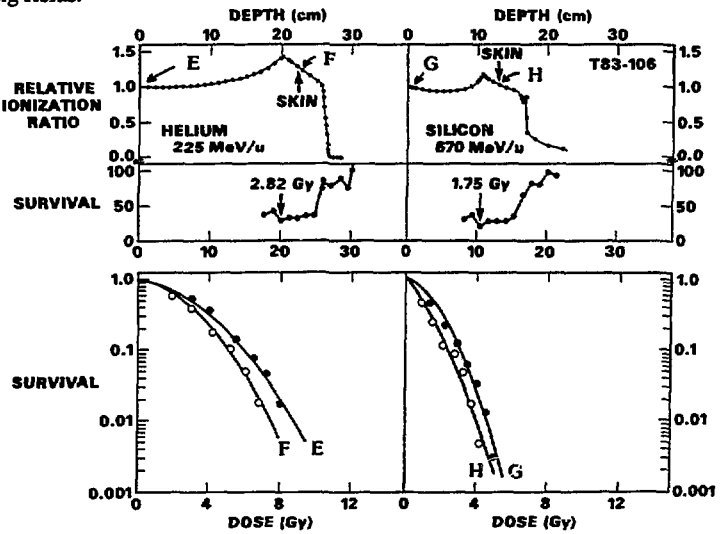

Fig. 3. Cellular survival measurements made at designated positions in the Bragg ionization curves of both a 225 MeV/u helium beam and a 670 MeV/u silicon ion beam using two biological techniques. (upper panel) Measured Bragg curves. (mid panel) Human T-1 cellular survival as a function of depth of penetration in water equivalent material after exposure to a dose fraction of 2.68 Gy helium or 1.58 Gy silicon ions. (lower panel) Survival curves measured at positions indicated in Bragg curves of each beam.

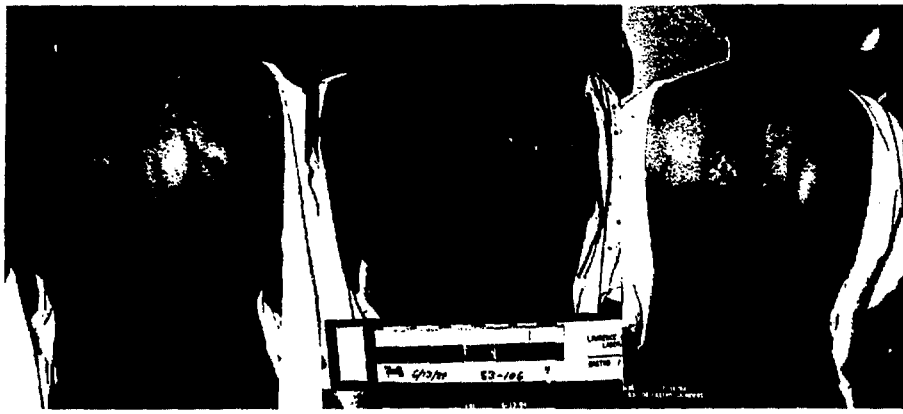

Fig. 4. Photographs illustrating skin reactions to doses of Bragg peak silicon ions and Bragg peak helium ions on adjacent fields on the last day of treatment (left), after three weeks (middle), and after two months (right panel). See text for further discussion.

studied is presented in Fig. 6. The electron response is shown on the right, with progressively steeper responses at lower dose fractions for helium peak, carbon peak and finally the steepest response was observed for Bragg peak neon ions. This kind of plot allows a RBE comparison in the middle of the skin scoring scale at the level of 4.5 - 5.0.

To put our data in perspective with some of the neutron RBE data, we have taken the plot of the RBE versus dose per fraction of 7.5 MeV neutrons by Field, *et al* (8) where a comparison is made of human skin responses at low doses per fraction to those obtained at higher doses per fraction in rat, pig and mouse skin.

Hopewell (9) has extended this analysis to higher energy fast neutron skin RBE values in the pig. The higher energy neutrons show a less steep slope than the 7.5 MeV neutrons. Only carbon and neon charged particle data have been added to this figure (shown as stars). The human skin data from carbon and neon peak exposures remarkably fall on the same line in this log/log plot. The three stars on the right of the lower line are from the fractionated carbon peak data of Leith, *et al* (10) with hamster skin.

The star with the filled symbol is the RBE value we have obtained in our analysis with 308 MeV/nucleon Bragg peak carbon ions on patient skin. The line of particle data lies lower than the 7.5

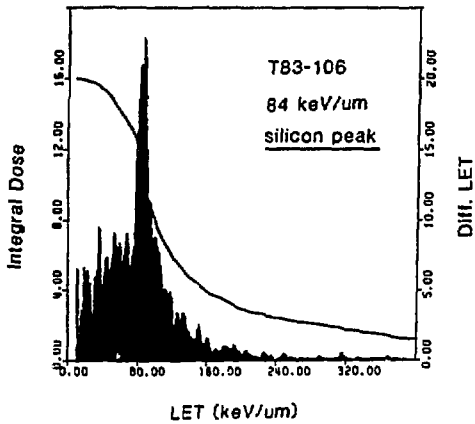

Fig. 5. Dose-average LET distribution from exposures of particle-sensitive CR39 plastic to the extended Bragg peak of a 670 MeV/u silicon ion beam. The peak LET value was 84 keV/μm in the Bragg peak as analyzed by E. Benton.

MeV neutron data line, but the low-LET reference was gamma photons for the neutron work, and was electrons or x-rays for the particle work. Correcting for this difference would shift the lower curve up slightly. The star with the open symbol is from our neon Bragg peak analysis. To our knowledge, there are no other available fractionated neon data from experimental animals.

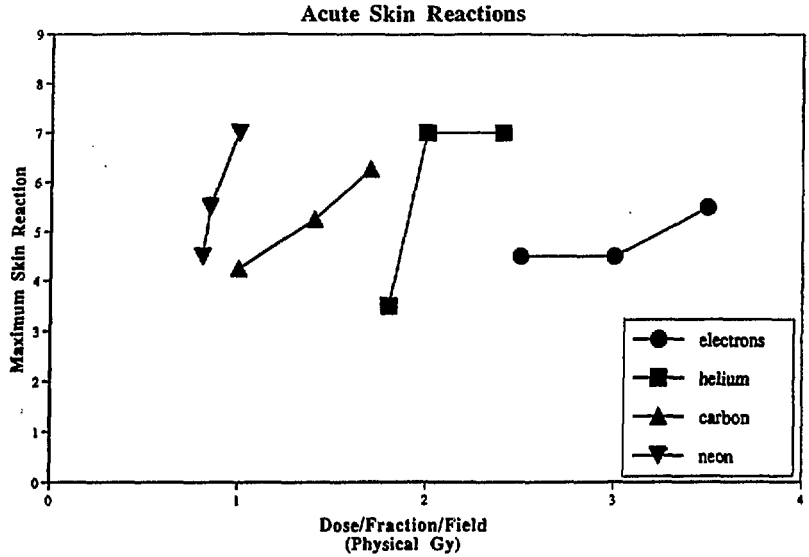

Fig. 6. Maximum human acute skin reaction versus dose per fraction per field of electrons, helium, carbon or neon ions.

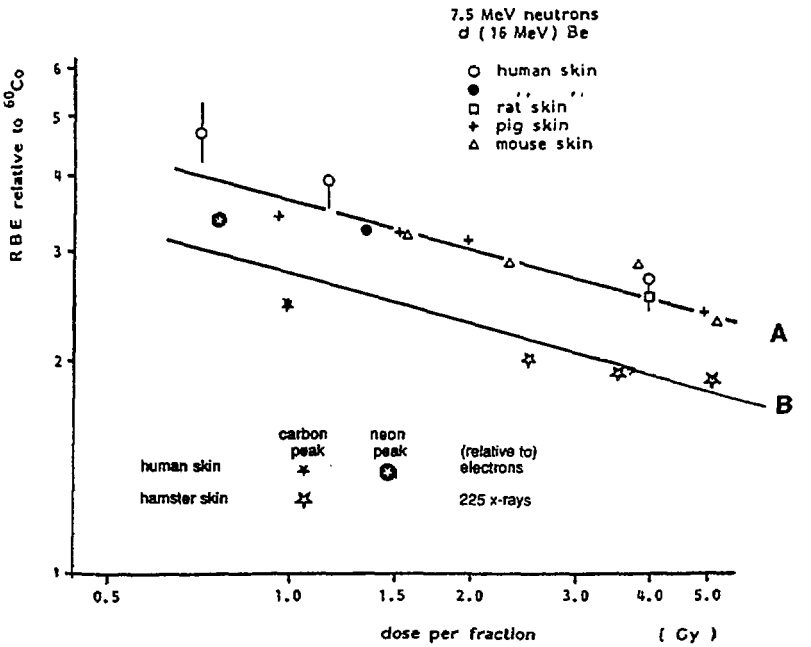

Fig. 7. RBE versus dose or dose per fraction for skin of different species, modified from Field, *et al* (8). Curve A represents neutron RBE data referenced to ^{60}Co radiation. Curve B represents carbon and neon RBE data referenced to electrons on xrays.

Data analysis

The dose-survival data were fitted to the linear-quadratic model of cell inactivation using programs written by Albright (7). These programs permit the calculation of radiobiological parameters and their 95% confidence intervals. The relative biological effectiveness (RBE) value for cell killing was determined from the ratio of the doses of helium Bragg peak ions to Bragg peak silicon ions 50% survival.

RESULTS AND DISCUSSION

Skin reactions

A compilation of the skin reactions to electrons, helium, carbon and neon is presented in Fig. 1. We detect dose-dependent shifts in the appearance and severity of the reactions on the 9 point scale covering 3 dose fraction sizes delivered in 10 fractions. We have emphasized the electron, helium, carbon and neon data because of its relevance to other ongoing programs. We do, however, have a small number of patients whose skin reactions were from heavier ions including silicon and argon. In that regard, one of the most dramatic tumor responses observed was in a patient treated with argon ions whose superficial tumor regressed significantly without any remarkable skin reaction. The data are obviously not statistically significant. However, we note that Blakely, *et al* (6) have previously observed in tissue cultures that argon beams reduce the radiobiological oxygen effect to a greater degree than any other beam studied is capable of doing. Further explorations with silicon or argon beams is clearly of interest to basic research.

To introduce the skin reaction data to heavier ions, we will describe a lung tumor patient whose multiple nodules were irradiated with an upper field of Bragg Peak high energy silicon ions, and whose deeper nodules were irradiated in a lower field with 225 MeV/nucleon helium ions (Fig. 2).

Radiobiology with a human fibroblast cell line in vitro was completed at the same time under the beam conditions of the patient treatment. The cell survival differences measured at the entrance and Bragg peak positions of each beam corresponding to the skin positions of interest are presented in Fig. 3. The right lower panel shows the enhanced killing of the silicon ions. The middle panel illustrates the confirmation of appropriate shaping of the ridge filters to yield isoeffectiveness at the dose fraction sizes selected.

A composite skin reaction photograph. is presented in Fig. 4. The photograph on the left was taken on the last day of treatment, The upper field was silicon, the lower field was helium. After three weeks, the middle photo, shows that the reactions were approximately equal, with perhaps the silicon field showing a little more color. However, at two months, the silicon field developed a significantly greater reaction and had a mottled appearance. It was difficult to assign a quantitative value to this acute skin response which included pigmentation changes suggestive of late skin reactions after low-LET radiations. The LET spectra of the silicon ion beam is presented in the lower panel of Fig. 5. The figure clearly indicates that the maximum dose-averaged LET number (84 keV/μm) actually represents a spread of LET values which complicates interpreting these data. Doses of plateau silicon ions with a significantly lower maximum dose-averaged LET value of 46 keV/μm did not cause this advanced late skin reaction in the single patient studied.

Table 2 indicates the relative silicon RBE values calculated from the *in vitro* cell experiments with Bragg peak silicon ions. The reference radiation was Bragg peak helium ions.

Table 2. RELATIVE SILICON RBE VALUES

	cells in vitro at 50% survival
Helium peak	
Silicon peak	3.2 ± 0.4

A preliminary analysis of the maximum clinical skin reactions as a function of physical dose per fraction per field

CONCLUSIONS

Carbon and neon skin reactions

We have begun a systematic reassessment of acute and late effects in cancer patients treated with charged particles. Preliminary comparisons indicate that Bragg peak carbon ions are slightly less effective than low energy neutrons (7.5 MeV). However, with normalization to similar low-LET reference radiations, carbon Bragg peak ions are nearly comparable to the neutrons. This conclusion about the acute effects of approximately 300 MeV/nucleon carbon ions is an independent qualitative confirmation of Ando's work with carbon ions presented at this Symposium. Bragg peak neon ions uncorrected for the RBE reference are more similar to the 7.5 MeV neutrons, which means that making this correction will shift the neon RBE to a value slightly more effective than the low energy neutron response.

Silicon ion skin reactions and radiobiology

Bragg Peak silicon ions demonstrate an enhanced acute skin reaction and a premature appearance of late effects, that perhaps speaks to the possibility of a significantly different biological mechanism of damage or repair. It is our opinion that normal tissue toxicities should be mapped out, port by port, with the physicians aware of critical normal tissue in each treatment field, and treatment planners accounting for tissue-specific, dose-dependent RBE values. This approach however will only be achievable when we have more information on the particle radiobiology of individual tissues.

Summary

High-LET charged particles have not had sufficient study to prove or disprove their merits in clinical therapy. However, despite promising tumor results (Linstadt, *et al*, 11, and Castro, 12), our follow-up studies have shown neon ions and silicon have had significant late effects on normal tissues. However, the observations presented in this paper should not necessarily mitigate against therapeutic applications of neon ions. Neon ions can provide deep tumor therapy with reduction of the radiobiological oxygen effect and of inherent radioresistance.

For the immediate future, we suggest that the carbon ion beam is to be preferred for further study of deep-seated tumors since it has biological dose localization advantages which are better than protons or neon ions. The ratio of dose in the tumor volume relative to the entrance region is maximized. A sufficient high-LET dose component is present to provide significant DNA damage, and suppression of radiation repair, maximized in the tumor because of the dose localization secondary to charged particles. Full use should be made of dynamic conformal therapy techniques to protect normal tissues and increase the dose in the tumor. We suggest that silicon ions would be advantageous for superficially-placed resistant tumors because of their high-LET advantage.

Techniques for predictive assays should be continued to be developed and tested in the clinic. These include tests of tumor growth kinetics, assays for inherent normal tissue and tumor radiosensitivity, assays of tumor hypoxia and assays to evaluate level and site of DNA damage. Combining these approaches may lead to individual patient profiles which will predict who might benefit from high-LET therapy, and how the selection of these patients should be made. A determined effort should be made to study tumor resistance at the genomic level and search for high-LET mechanisms that overcome this resistance. We anticipate that further follow-up and study of the patients treated with neon ions at LBL will contribute significantly to the rational use of heavy charged particles in the future.

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