MESON RADIOBIOLOGY AND THERAPY

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Abstract

High-linear energy transfer radiation (neutrons, heavy ions, and pions) have a greater relative biological effectiveness than low-linear energy transfer radiation by depositing a high density of ionization in irradiated cells. This overcomes the protective effect of oxygen; decreases the variation in sensitivity among the several stages of the cell cycles; and, inhibits the repair of sublethal damage as compared to x-rays, gamma rays, electrons and protons. Negative pi mesons (pions), appear particularly suited for radiation therapy as their penetration and depth-dose pro-file lend themselves to shaping the high dose area to the tumor size and location. Preliminary biological experiments with pions produced at the Los Alamos Meson Physics Facility studied cell survival at various radiation depths and cell cycle sensitivity. Histologic study of data from the first human experiments indicated severe tumor cell destruction by pions as compared to x-rays in treating malignant melanoma skin nodules, without increased effects on dermal elements.

1. <u>Differences in Biological Response to</u> Radiation

1.1 Relative Biological Effectiveness

The microscopic deposition of energy in tissue molecules by radiation is referred to as linear energy transfer (LET). Energy deposition occurs in the form of ionization. A low-LET beam passing through a volume of tissue results in relatively few ionization points as compared to the densely ionizing path of a high-LET beam. The degree of cell damage is directly related to the ionization density produced by the radiation. Cells are unable to repair molecular injuries caused by high-LET radiation as well as they can repair injuries caused by low-LET radiation. Thus, high-LET radiation is said to have greater relative biological effectiveness (RBE) than low-LET radiation due to the higher number of ionizing events per microgram of tissue.

This high density of ionization caused by high-LET radiation alters certain classical biological responses. Generally, normal cells have a greater capacity to repair themselves than do tumor cells arising in normal tissue. The increased ability of normal cells to repair sublethal damage makes cure by radiation possible, since it is known that the sensitivity of normal and abnormal cells to conventional low-LET radiation is about the same. Multiple small daily fractions of x-ray (<200 rads) permits a greater degree of repair to occur in normal cells than in abnormal cells in a specific time The reduced amount of repair in period. normal tissues that occurs with high-LET radiation mitigates against the favorable effects of fractionation and can be a disadvantage in the clinical situation.

Radiation beams exhibit characteristic penetration profiles. Gamma rays, x-rays, and neutrons deliver the highest radiation dose near the surface of the body; the dose decreases exponentially as it passes through the tissues. Beams of heavy charged particles such as neon, helium, protons, and pions, deliver their dose at a finite depth which is proportional to the energy of the beam.

The absorption curve for heavy charged particles has two distinct regions; initially, the particle travels along a lowdose plateau. As the particles in the beam lose energy, decelerate, and stop, a proportionately greater amount of energy is absorbed per unit volume of tissue, thus creating the high-dose peak, the Bragg peak or stopping region. The initial energy of the radiation beam governs the depth of the peak below the body surface.

In radiation therapy, the idea is to fit the beam as closely as possible to the size and shape of the tumor. Radiation from conventional supervoltage x- and gamma rays or from neutron beams cannot be restricted to the tumor volume. To avoid giving an excessive dose to nearby normal structures, it is often necessary to lessen the dose to the tumor itself. Theoretically, heavy charged particle beams can be shaped to fit a tumor by regulating the energy and shape of the beam so that the peak dose coincides with the location and shape of the tumor. Since the amount of energy deposited in adjacent normal tissues can be minimized, it should be possible to deliver a higher dose of radiation to the tumor itself. This increases the probability of destroying all tumor cells and, hence, the probability of cure.

Among the charged heavy particles, pions and protons show the most advantageous peak-to-plateau ratio in their absorption curves. Although heavy ion beams have an excellent peak-to-plateau ratio, they have a narrow peak, which is better suited for treating small volumes of Larger tumors which are not well tissue. managed by conventional means require a wide spread in the peak region of the beam. Scanning the heavy ion peak across a large tumor deposits a larger amount of plateau low-LET radiation in the tumor, thus reducing the peak-to-plateau ratio. Since pions have a wider peak than heavy ions, scanning does not reduce the deposition of high-LET radiation in the tumor region to the same degree. Theoretically, then, pions would appear to be more advantageous than heavy ions for management of large tumors. While protons have a peak-toplateau ratio similar to pions, they do not have the advantage of high-LET radiation in the peak region.

The negative pi meson, or pion, is a subnuclear particle with a mass approxi-mately 1/7 that of a proton. In the plateau region of the absorption curve, it behaves as low-LET radiation. In th In the peak stopping region, it yields a high-LET dose. Two occurrences account for the high absorbed dose in the pion stopping region. First, as the pions slow down and stop, there is increased ionization per microscopic volume because the pion is in the volume for a longer time. Second, as the negatively charged particles lose energy, they are captured by the nuclei of carbon, nitrogen, and oxy-gen. This additional charged mass causes an unstable condition in the nucleus of the atom resulting in nuclear fission with the production of alpha particles, large nuclear fragments, neutrons, and a small percentage of gamma rays. The ionization pattern of the fragmentation, called the star, is the high-LET portion of the pion beam and it augments pion radiation energy in the peak region.

Figure 1 shows the depth-dose profile of the pion beam used in the first biomedical experiments at the Los Alamos Scientific Laboratory's Meson Physics Facility, as measured in the fall of 1974 1). Note that the pion peak includes both high- and low-LET radiation. This tuning configuration sacrifices some sharpness in the definition of the peak to obtain the increased dose rate required for biological experiments.



Figure 1. Depth-dose profile of LAMPF pion beam for initial biomedical experiments. (Reprinted with permission of <u>Radiology</u>.)

We have so far discussed the greater biological lethality of high-LET radiation over low-LET radiation. We have noted that normal cell repair is also inhibited by high-LET radiation but, with pions and heavy ions, this is mitigated by the capacity to confine the high-LET dose to the tumor volume. In particular, we have seen that pions have an absorption curve which may especially suit them for treating large, deep-seated, irregularly shaped tumors.

1.2 Cell Radiosensitivity

Biological effects of radiation are not solely determined by density of energy deposition and dose. In addition, radiation protection resulting from a lack of oxygen in the tumor or related to cell cycle phase must also be considered.

1.2.1 <u>Hypoxia</u>. Most tumors have microscopic areas where cells are hypoxic. Normal oxygenated cells are more sensitive to radiation than hypoxic tumor cells. Hypoxic cells may require up to three times as much radiation as well-oxygenated cells for destruction. For a given type of radiation, the ratio of hypoxic cell to normal cell radiation sensitivity is called the oxygen enhancement ratio (OER). The smaller the OER, the less hypoxic tumor cells are protected from that type of radiation and the more nearly hypoxic tumor cells react like oxygenated tumor cells to radiation delivered.

Hypoxia gives less cell protection against high-LET radiation than against low-LET radiation. Thus, neutrons and pions, for example, have a lower OER than x- or gamma rays. According to Withers ²) hypoxia probably does not affect the

radiocurability of many human tumors because the application of many low-dose fractions causes most types of tumors to respond as though they were completely oxygenated. Based on experiments using clinical fractionation schedules of six to eight weeks, Withers theorizes that since well-oxygenated cells are more radiosensitive, they are killed early in the course of a fractionated dose regimen. Neighboring hypoxic cells then have better access to the available oxygen and become reoxygenated. Hence, their radioresistance changes to radiosensitivity by the time the next dose is given. Small doses of radiation per fraction essentially restrict killing to the oxic cells. Dose is not wasted on anoxic cells that will become oxic later in the course of therapy.

Withers describes the greater effectiveness of high-LET radiation in tumor cell destruction due to decreased OER as the therapeutic gain factor (TGF). The TGF depends on the percent of dividing tumor cells that are hypoxic and on the size of the radiation fraction dose. High-LET radiation results in a therapeutic gain if large single doses (1,000 rads) are The larger the percent of hypoxic used. cells, the greater the therapeutic gain. The TGF is essentially non-existent when small daily doses of radiation are given, or if there is a low percentage of hypoxic cells in the tumor. Since fraction size is small in clinical treatment, hypoxia probably does not affect radiocurability of many human tumors, although the response of some large tumors may be determined by hypoxic cells.

Raju and Jett compared the survival of T-1 cells irradiated with high-LET radiation under conditions of hypoxia and oxygenation 5). To simulate the high- and low-LET mixture of the pion peak, they used different quantities of x-rays and high-LET radiation, the latter from alpha particles emitted by 239 plutonium. They found that only a small proportion of high-LET radiation was necessary to reduce the OER. However, the RBE increased almost linearly as the proportion of high-LET radiation increased.

1.2.2 <u>Cell cycle</u>. The sensitivity of a cell to radiation also varies according to its stage in the cell cycle. With some exceptions, mammalian cells are usually sensitive in the mitotic phase and resistant in the late DNA synthetic phase 3). At doses used in clinical radiotherapy, the variation may be greater than the variation between oxic and hypoxic response ²). However, the variation of radiosensitivity across the cell cycle is reduced considerably by high-LET radiation. Studies have indicated that radiosensitivity variations due to cell cycle can be observed for highLET radiation only with highly synchronized cells irradiated with high doses and with a high-LET component greater than 100 keV/micron $^{5)}$.

If there were a larger proportion of tumor cells than normal cells in radioresistant phases of the division cycle, high-LET radiation would be relatively more damaging to the tumor than x-rays However, the converse would make high-LET radiation therapeutically disadvantageous. Since these cyclic fluctuations in radiosensitivity are less with high-LET radiation than with x-rays, it is likely that variations in response from tumor to tumor would be less than are found with x-radia-This would result in steeper tumortion. control probability curves, which would be more dependent on dose variations. Thus, accurate dosimetry and treatment planning, as well as the requirement that treatment be taken to the full tolerance of normal tissues, become more critical. Because of the oxygen and cell cycle effects, high-LET radiation may be most useful in treat ing large tumors that are either partially hypoxic or that differ markedly from adjacent normal tissue in degree of proliferative activity. Examples are glioblastomas and osteosarcomas, which rapidly outstrip in growth the adjacent tissues

2. Pion Biological Experiments at LAMPF

Pions, uniquely, appear to have the advantages of both high- and low-LET radia-A program to test the biological tion. and clinical effects of pion radiation is underway at the Los Alamos Meson Physics Facility (LAMPF) as a joint effort of the University of New Mexico Cancer Research and Treatment Center and the Los Alamos Scientific Laboratory. The studies are supported by grants to UNM by the National Cancer Institute, and funding from the United States Energy Research and Development Administration for operation of the biomedical channel. Physicians from some 20 institutions throughout the United States are participating in the planning for the clinical trials at LAMPF, and will send appropriate patients for study.

Pions were obtained in the biomedical research area on February 6, 1974, and the first radiobiology experiments began in June of 1974. Pions are produced at LAMPF by a half-mile-long linear accelerator which is designed to accelerate protons to 800 MeV at an average beam current of 1 milliampere. For this initial run, the accelerator was operated at 10 microamperes. The current was deliberately kept low so that induced radioactivity in the accelerator tunnel would not inhibit correction of problems identified during the initial run. Priorities for preclinical experiments have been based on the need for biological information preparatory to clinical trials. Initial preclinical studies included cell biology experiments to establish a depthdose biological effectiveness curve. Although preliminary microdosimetry studies indicated the location of the high-LET pion region, it was expected that complete microdosimetric data would be developed over a considerable period of time.

Two of the biology tests which were conducted at LAMPF using the pion beam have particular implications for patient treatment. Todd <u>et al.</u> studied the survival of cultured human (T-1) cells at various radiation depths. Raju <u>et al.</u> investigated cell survival as a function of depth and oxygenation.

In his experiments, Todd $^{4)}$ utilized a series of glass coverslips to which cells from the human kidney were attached. The coverslips were placed 3 mm apart in a water-tight box which was submerged in a water phantom with the central axis of the beam perpendicular to the coverslips. Peak pion doses from 100 to 400 rads were then delivered. With 400 rads, approximately 30 percent of cells survived in the region immediately distal to the total maximum peak dose, compared with 75 percent for a dose of 100 rads. Since it is advantageous to fractionate x-rays in cancer therapy to maximize recovery, one experiment examined recovery with pion radiation. Four doses of 100 rads over nine hours were compared with a single dose of 400 rads of peak pions. The cells that received 400 rads in 100-rad installments survived as if they received 340 rads in a single dose.

Raju et al⁵⁾ used gelatin to suspend human kidney cells for exposure to pions, to study cell survival as a function of depth, and Chinese hamster ovary cells (CHO) suspended in sealed pippettes to study cell survival under oxic and hypoxic conditions. The RBE at the peak was higher than that at the plateau by a factor of 2.0, and the oxygen enhancement ratio was 1.5 at the peak position.

3. Pion Human Biology Tests at LAMPF

After some preliminary mammalian tests, human radiobiologic experiments using metastatic tumor nodules in the skin and surrounding normal skin were begun 6). The objective was to observe and compare the responses of the epithelium and the subcutaneous tissues in the pion stopping region with responses of those tissues to x-radiation. A second objective was to obtain information on the relative response of human tumors to peak pions as compared with x-rays.

Since safety was the principal restriction in planning these first human experiments, the tolerance of the normal tissues to superficial x-rays was used as a guide to dose levels. Tests began with doses of 60 to 75 percent of skin tolerance for superficial x-rays, fractionated over an eight-day period. When no untoward acute effects were seen in the skin, a test was conducted to establish a dose-response curve for peak pions in the high-LET region as compared with 140 kVp x-rays.

The subject for this experiment was a single patient with malignant melanoma with multiple skin nodules. Eighteen nodules between 5 and 16 mm in diameter were randomly assigned to six groups of three nodules each. Three groups of nodules were given total surface x-ray doses of 55, 66, and 75 percent of 5,200 rads, in 15 fractions over 19 elapsed days. The other three groups were treated with peak pions at 50 percent of the x-ray doses at each of the three levels with the same fractionation schedule.

The x-ray machine was operated at 140 kVp with a half value layer of 2.6 mm aluminum. The dose rate was 500 rads per minute. The peak pion dose rate varied from five to seven rads per minute. A 3 cm diameter collimator was used for all pion-treated skin nodules and all x-raytreated nodules save one in the 55 percent group which required a 5 cm collimator. The dosimetry performed prior to each irradiation was sufficiently precise to allow placement of the beam, with respect to the surface of each collimator, within a fraction of a millimeter. The change in dose as a function of depth below the collimator was similar for x-rays and pions to depths of at least 0.5 cm. The beam profile across the 3 cm collimator was essentially flat for x-rays over a central diameter of 2.3 cm, but approximately Gaussian for pions, with a full width at half maximum of about 3 cm.

Color photographs were made at least twice a week of each of the 18 irradiated areas using constant lighting and exposure factors. Optical density was measured from the photographs, using a McBeth densitometer, Model 504, with Kodak filter 58 (green). The combination of film (high speed Ektachrome Tungsten with 85B filter) and densitometer filter provided optical density measurements which increased the degree of erythematous skin reaction. The skin adjacent to each treatment area was adjusted to zero on the densitometer, and the optical density of the treated area was measured. Dose response curves were obtained by plotting average values for the maximum skin effect and calculating a least squares fit. These curves were linearly interpolated to determine the pion and x-ray doses that would produce an optical density of 0.22, and the ratio of these doses was used to calculate skin RBE.

The clinical observations of the radiation therapists and the optical density measurements indicated that maximum erythema occurred during the fifth, sixth, or seventh week after treatment started. The ratio of doses obtained by averaging the optical densities resulted in a radiobiological effectiveness (RBE) for skin ery-thema of 1.5 \pm 0.3 at the optical density of 0.22. At all dose levels, skin reactions due to x-ray were observed as more intense than those produced by pions. How-ever, surface dose distribution across the 3 cm x-ray field was greater than 90 percent in the central area of 4.15 cm^2 , while the central high dose area of the pion beam was only 1.63 cm². This decreased area of high dose for pions as compared to x-rays may have contributed to a decrease in maximum reaction in the pion fields. No unusual untoward effects were observed in the normal skin or subcutaneous tissues in any of the patients treated in this series of tests after four and one-half to six months observation.

In the opinion of the radiation therapists, the tumors treated with pion radiation regressed at least at the same rate as those irradiated with x-rays and the nodules became softer somewhat more quickly, which corresponds to observations made in earlier mammalian tests. However, information on regrowth was not adequate for calculation of tumor RBE prior to the patient's death from generalized disease, 24 weeks after the tests began.

At no time before death were signs of dermal thickening or thinning noted. Each nodule area with a generous untreated skin margin was excised and microscopic sections were prepared. None of the pionirradiated nodules studied to this point contain identifiable melanoma cells, except one with cells that appear to emanate from regrowth of a nodule adjacent to the one treated. The x-ray-treated nodules all demonstrated identifiable and apparently viable tumor cells 7).

Figure 2 is a low-power photomicrograph of a peak-pion-treated area. The epidermus, although thin, is little different from adjacent untreated skin. Extreme accumulations of dense melanin pigment granules have been found, either loose within tissue or possibly present within the cytoplasm of histiocytes. The letter C marks an inclusion cyst resulting from the original growth of melanoma squeezing off the neck of hair follicles and sweat glands. These cysts persist after tumor is destroyed. No tumor cells are seen.



Figure 2. Lesion treated with 75% pion dose. Beneath an intact epidermis (top) coarse accumulations of dark pigment scattered within the dermal collagen. Pigment corresponds to melanin liberated from necrotic melanoma cells. Small keratinous cyst (C) is believed to be secondary to previous occlusion of a hair follicle by masses of melanoma cells (now necrotic). H and E x 40.

Figures 3 and 4 are high-power photographs of the same peak-pion-treated area. No tumor cells are seen. Melanin is concentrated around dermal appendages (G) and around nerve twigs, the places where tumor grew luxuriously because of increased vascularity around these structures.

By contrast, Figures 5 and 6 respectively are low and high power photographs typical of x-ray-treated nodules. Arrows point to viable tumor. At all dose levels,



Figure 3. Higher magnification of accumulations of melanin pigment surrounding sweat glands (G). Finely granular pigment is largely present within histiocytes. No identifiable remnants of viable tumor cells. H and E x 360.

viable tumor is present. To estimate the relative effect of pions and x-rays in normal tissues, a Chalkley count was performed on the dermis of control (i.e., untreated) skin, x-ray-treated skin, and pion-treated skin. Two specimens of each were quantitated. Each of the six specimens was counted three times, 100 counts each time, and the mean from the separate examinations of each tissue recorded. Table I illustrates that the mean of each type of cell or structure is similar for all specimens. Therefore, at 24 weeks, no increase in normal tissue effects was seen with peak pions as compared to x-rays, even though total tumor destruction was accomplished by the pions. Therefore, at this intermediate time after the acute normal tissue reaction had subsided, at x-ray dose levels 55, 66, and 75 percent of tolerance, and with physical peak pion doses numerically one-half of the x-ray doses, normal tissue effects are simi-lar in both types of treatment without significant change from untreated sites. However, complete destruction of melanoma cells is apparent with peak pions even at the lowest dose levels.

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Figure 4. Accumulation of melanin around nerve twigs in pion-treated area. No tumor cells. H and E x 360.

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Figure 5. Beneath an intact epidermis a nodular accumulation of dark pigment corresponds to melanin presumably liberated from necrotic melanoma cells. However centrally within this area (arrows) there are intact tumor cells as well. H and E x 40.

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Lesion treated with 75% x-ray dose. Figure 6. Higher magnification from area indicated by arrows in Figure 5. Melanin pigment within histiocytes or loose within stroma is evident at the periphery while centrally apparently viable melanoma cells remain (arrows). H and E x 340.

TABLE I

NORMAL TISSUE ELEMENTS

	Collagen	Capillary; Venule	Artery; Arteriole	Dermal Appendage	Artifacts; Tissue space
<u>Skin Site</u>					
Control	89	6	<1	1	4
X-ray	87	8	1	<1	3
Pion	84	8	<1	2	5

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DISCUSSION

M. EDER: Did the success of pion treatment of tumors have any connection with how the tumor originated?

M. KLIGERMAN: There was no connection with how tumors arose and the results.

R. WIDERÖE: I suppose that you treated the skin and the tumors at the peak of the pion depth dose curve?

M. KLIGERMAN: Yes.

T. KUO: What is the low LET component of the dose curve?

M. KLIGERMAN: The low LET component is due to the ionization of the primary pion beam. The high LET component is the result of nucleon capture of the pion in the peak region when its energy is low. Capture by the nuclei of C, $N_2 + O_2$ causes an unstable condition of the nucleus, with fragmentation into alphas, neutrons and other large nuclear fragments. which behave as high LET.

T. KUO: In your statement of skin reaction, the X-ray dose is twice that of π^- . What is the normalization factor?

M. KLIGERMAN: Using the optical density as a measure, peak pions were 1.5 times as effective in causing the same level of erythema in the skin as 140 kV X-rays.