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RESEARCH PROPOSAL

LOS ALAMOS MESON PHYSICS FACILITY

PION RADIOBIOLOGY

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SUMMARY OF EXPERIMENT

The objective of this project is to provide a broad base of information on the expected responses of normal and tumor tissues following exposure to negative pions so that clinical trials of pion radiotherapy can be designed most effectively. Biological studies will be conducted to determine injury to normal and tumor tissues in the peak pion region, the sparing factor for normal tissues adjacent to the tumor volume and/or in the plateau beam path; optimal fractionation schedules; isoeffect characteristics; and reasons for observed changes in therapeutic effect.

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PROPOSAL INFORMATION

Beam Area: A East

Secondary channel: Biomedical channel

Beam requirements: Therapy π^- beams

Primary beam requirements: $> 20 \mu\text{A}$

Running time required (per year):

Installation: 200 hours

Tune-up: 100 hours

Data runs: 600 hours

Scheduling: In blocks of ~ 100 hours after primary beam current of $20 \mu\text{A}$ is achieved

Major LAMPF apparatus required: None

Shielding and enclosures required: None

Special services required: None

Space required: as available

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REPORT

To decrease the time to gather biological data before safely embarking on clinical trials, certain of the experiments must be designed without all of the requisite information. This has tended to broaden the range of dosas or other conditions studied, but the additional experimental effort required is considered small relative to the time which will be saved. In certain of the experiments, experimental methods are flexible and final decisions regarding the methods will be made on the basis of small scale experiments which are presently in progress. We have not examined all of the options available for each experiment, but list our choices in terms of available assays. Finally, a number of the experiments offer the possibility of additional information, e.g., salivary gland analysis in animals given total brain irradiation for the histologic analysis of CNS injury. Again, these aspects of the design are left flexible and will be performed if time or consultant collaboration is available.

The preclinical biology studies can be divided into: (a) analyses of normal tissue responses to x-rays and peak pions at equally tumoricidal levels, and (b) biological experiments related to beam development, mechanism studies, and development of rapid assay systems.

a. Comparative Normal Tissue Injury at Equally Tumoricidal Levels of Pion and X-rays

These experiments are designed to determine the relative injury to a variety of normal tissues within the target volume when equally tumoricidal doses of pions and x-rays are employed. Here we are determining the relative effects of peak pions and x-rays on normal tissues immediately adjacent to the target tumor. The sparing effects of pions in the normal tissues outside the target volume but within the path of the beam are addressed later in this section. Because of the dose distribution of pions, it is likely that the normal tissues within the target volume will be the limiting factor in therapy; therefore, a full understanding of the pion versus x-ray dose response curves for these tissues is a requisite for clinical application.

These experiments are designed to analyze each endpoint in mice/rats that have been given graded doses of pions or x-rays in 1, 2, 5, or 10 fractions. (When appropriate and feasible, 15 fractions will be used to simulate the clinical setting.) The dose ranges used will vary as a function of the endpoint under study and the fractionation schedule for both types of irradiation. The range will be chosen to bracket the 50% response point (or the midpoint of the observable range in quantitative studies). Involved in these calculations are an expected progression of the relative biological effectiveness of pions from 1.4 to 2.0 as the fractionation increases from 1 to 10 fractions (rapidly dividing tissues), and an expected reconstitution of a 300-rad shoulder for the x-ray exposures in the other tissues. The dose range which brackets the expected 50% response point could be off by a factor of ± 2 and still allow estimation of the 50% response. For each tissue a pilot group of animals will be exposed to a dose of radiation which is known to yield 100% responses (e.g., 10,000 rads of x-rays for the induction of spinal cord injury) so that the rate of injury progression can be ascertained prior to analysis of the main portion of the experiment. Pilot experiments are also in progress to determine whether the rate of injury appearance is dose dependent.

(1) Normal Tissue Studies

The following proposed studies represent a broad spectrum of tissue types expected to be irradiated in clinical tests.

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(a) Brain

The endpoints available for analysis of radiation injury to the brain are limited and fall into one of two categories: near immediate (<24 hours) shock-type death following the administration of massive doses (>>10,000 rads), or long-term histopathologic evidence of injury. Since the former has little clinical relevance and yields anomalous recovery artifacts (25) the present studies will concentrate on long-term histologic evidence of injury.

For this endpoint, and for all others, the dosimetry will be performed for the site in question. Based on the unknowns involved in dose distribution in inhomogeneous absorbers, this individualized dosimetry is considered an important aspect of the experiments. C₃H mice will be given total brain exposures with no attempt to shield the salivary glands due to their relative radiation resistance in this species (26). The high dose group (10,000 rads of x-rays or its equivalent) will be killed at monthly intervals and sections of the brain examined for evidence of radiation injury. Unless these studies indicate that an earlier sacrifice date would be appropriate, the mice from the main portion of the experiment will be killed at one year and examined. Although skin studies will continue (using the mouse foot system described in Section A), the skin reactions in this and other normal tissue groups will also be monitored for three reasons. A much broader range of doses and exposure conditions will be available. These studies will provide an internal biological control for inter-comparison of the different normal tissue experiments. Confirmation and repeatability of the assays can then be estimated. As time permits, samples of the salivary glands will be examined by Dr. Marvin Sodicoff (Temple University) for evidence of morphological and functional injury.

(b) Spinal Cord

Rats will be exposed to graded doses of pions or x-rays with the exposure field being a 6 x 12 mm port encompassing the lumbar region of the cord. Following exposure, the rats will be checked weekly for evidence of paralysis and will be killed when moribund. The high dose group will be killed at monthly intervals and both unirradiated and irradiated portions of the cord examined for evidence of histologic injury. Exploratory collagen assays will also be performed in this group in collaboration with Dr. R. L. Ullrich, Oak Ridge National Laboratory, to quantitate connective tissue infiltration into the irradiated area.

(c) Colonic Mucosa and Supporting Tissues

The colon presents a unique problem in that preliminary experiments indicate that injury to the mucosa develops and heals very rapidly (less than one week), while injury to the supporting tissues, which is probably more critical for long-term clinical complications, requires more than a month to appear. This study will address the problem in terms of histopathologic examination. Experiments to be performed with Dr. H. R. Withers (see Section F) will address the radiation effects on the small intestines in terms of "clonogenic unit" survival.

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The peak denudation of the colonic mucosa occurs three to four days after exposure, while the peak manifestation of the injury to the supporting tissues occurs between one and two months after exposure. Therefore, the size of this experiment will be doubled with lower dose groups killed within the first week for analysis of mucosal injury, while the higher dose group will be killed at two months for analysis of supportive tissue injury. A grading system has been developed for this type of injury which allows construction of dose response curves in terms of constriction of the colonic lumen as well as gradation of injury to the support tissues themselves.

(d) Heart

Based on reports in the literature and consultation with a number of experts, it appears unlikely that this experiment can be performed in other than the rabbit. We propose, therefore, to limit sample sizes to three rabbits per point and perform the same overall experiment as outlined above for the other tissues. Two endpoints will be employed in the pilot study on the high dose group: assay of ^{125}I UDR incorporation into the capillary endothelium and histologic examination of the later developing fibrosis. Since incorporation precedes fibrosis, we will choose between the two, based on our preliminary experiments, and select the one which allows most accurate delineation of dose response curves.

(e) Kidney

Pilot experiments, conducted in mice, failed to demonstrate radiation-induced lethality due to kidney exposure such as has been reported by Phillips. Raising of the radiation doses above 2,250 rads, which was suggested by Phillips as being the LD₅₀ for this type of death, did induce lethality but death was due to intestinal injury. We propose, therefore, to perform the same type of experiment (expose the remaining kidney one month after unilateral nephrectomy), but to concentrate on histologic examination of kidney injury as the endpoint. The difficulty of this approach lies in the excessive amounts of time required for injury expression, i.e., 12-18 months. Studies are in progress which attempt to develop quantitative histologic methods to detect injury prior to the onset of overt signs of renal complications. These include semi-quantitative analysis of glomerular changes and biochemical analyses of renal function. Further, two approaches to functional assays of kidney response are being evaluated: the ability of an irradiated kidney to hypertrophy following the removal of the unirradiated kidney and determination of the hypertrophy-atrophy response in unilaterally irradiated animals. Should any of these methods prove to be more adequate for the construction of dose response curves, the long-term histologic studies will be reduced in size but retained for the analysis of long-term degenerative and malignant alterations. In addition, we will also be examining the effects of irradiation after unilateral nephrectomy within time periods of a few hours to a few days, in conjunction with a parallel study now in the pilot stage. This information could prove useful in the clinical setting when patients with pelvic tumors exhibit evidence of metastatic spread to both kidneys.

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(f) Lung

The analysis of the response of the lung to pions and x-rays will involve a serial sacrifice study with the response endpoints being collagen deposition and histologic examination. Collagen analysis will be performed in collaboration with Dr. R. L. Ullrich (Oak Ridge National Laboratory). Only a portion of the left lobe of the mouse lung will be exposed in order to simulate clinical conditions and to avoid the mouse's acute lung death syndrome which is unlike the clinical problem in that it does not involve collagen deposition (27) but does involve edema and infection (28). Starting at six months after exposure and at three month intervals after that, the mice will be killed and the irradiated and unirradiated lobes weighed, and divided for histologic and biochemical analyses. Again, pilot experiments underway will be used to establish exact times of sacrifice.

(g) Skin

The major effort for analyses of skin injury will be conducted using the mouse foot system developed by Field et al. at Hammersmith, and tested under this program with x-radiation in preparation for a more intense pion beam. As pointed out above, additional skin analyses for x-rays and plateau pions will be performed as an adjunct to other organ studies.

(2) Tumor Response Studies

This experiment will provide a comparison of normal tissue injury (cost) versus therapeutic effect (benefit) for pions and x-rays. Two tumor systems will be employed to provide estimates of the therapeutic benefit: treatment of growing lung tumor colonies produced by the intravenous injection of tumor cells and treatment of intramuscular implants of the same tumor. For both types of assays, either a fibrosarcoma or a mammary tumor of the C₃H mouse will be employed. The choice between the two will be determined by pilot studies designed to estimate the more stable of the two.

In the lung colony assay system, mice will be given a sufficient number of cells intravenously such that 40 to 50 lung tumor colonies will develop (determined from pilot experiments). At seven days after transplant, when the tumor colonies average 100-300 cells per colony, the mice will be exposed to graded doses of pions and x-rays, and 21 days after the completion of therapy the mice will be killed and the number and size of colonies determined (29) according to standard techniques.

In the intramuscular transplant system, the same tumor (10^6 cells) will be transplanted and allowed to grow to a volume of 250-300 mm³, at which time exposures will be given. Following therapy, mice will be observed daily and tumor regression and recurrence noted. At least once a week the tumor sizes will be determined. All mice dying during the experiment (120 days) will be examined for tumor at the transplant site and metastatic spread.

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These two tumor systems in combination offer a standardized system for assessing the effects of pions and x-rays on rapidly growing marginally hypoxic tumors (lung tumor colony system) and more slowly growing tumors with a developed hypoxic fraction. More complete studies on the role of these two variables in the tumor response are discussed later in this section.

(3) Analytic Methods

The overall design of these experiments involves two types of radiation, two types of responses (normal and tumor tissues), and four fractionation schedules. While the overall aim is to express normal tissue injury versus tumor response (or vice versa), this design also allows estimation of recovery rates, dependence of RBE on dose size (when final rad estimates for pions become available), and a variety of other characteristics which are critical to the clinical application of pions.

Essentially two types of data will be obtained from these experiments: quantal and quantitative. For certain responses, such as skin injury, analyses may be performed via both methods. Again this will provide internal checks on our system and will provide the ability to address more subtle questions.

For quantal data (percent responders versus dose) probit analyses will be employed, but the comparisons between groups will not be restricted to the 50% response point. Figure 5 demonstrates the reason for this requirement. In both parts a and b, fractionation has had the same effect on the 50% response point, but in part a the effect of fractionation is total dose (and, therefore, fraction size) independent, while in part b the effect of fractionation varies with the size of the total dose. Analyses such as these will be required for the rational design of the clinical protocols.

Quantitative data will be handled in roughly the same way, i.e., with the analysis including consideration of the possibility of variable fractionation effects depending on dose size.

Finally, for the comparison of normal and tumor tissue responses we will be able to compare tumor injury as a function of observed level of normal tissue injury or as a function of fractions of the radiation dose required to produce a given level of injury. While the former is more accurate, the latter provides an approach which parallels the methods used in clinical planning.

b. Development, Mechanism, and Baseline Studies

The experiments described above will provide estimates of the injury that will occur following pion or x-ray treatment in those tissues which must be included in the treatment volume. They do not address the question of what is likely to be the major factor contributing to a therapeutic gain for pions: namely, the favorable dose distribution which spares normal tissues outside the treatment volume, henceforth referred to as "beam path tissues." To estimate the extent of sparing to these beam path tissues, it will be necessary to know the spatial distribution of dose along the beam path, the relative biological effectiveness as a function

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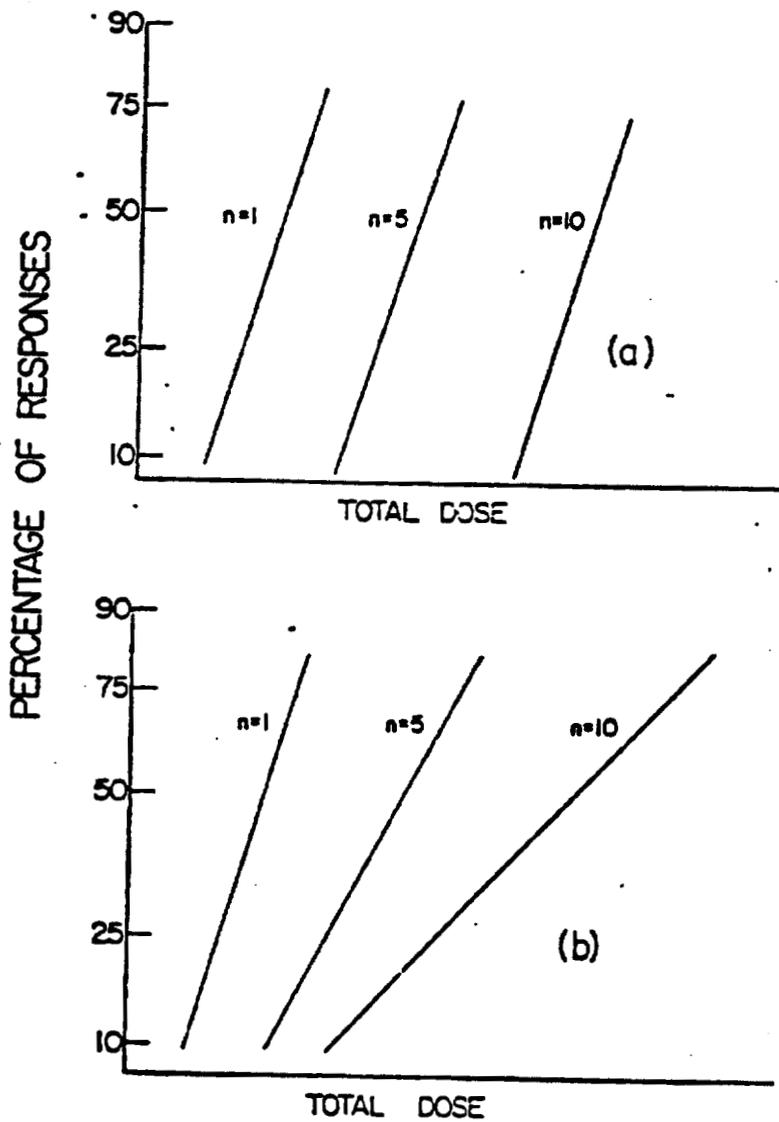


Figure 5: Quantal responses as a function of total radiation dose for 1, 5, and 10 fraction regimens: (a) recovery independent of fraction size, (b) recovery directly proportional to fraction size.

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of position in the beam (x, y, and z) and how the RBE varies with oxygen concentration under the same conditions. Experiments relating to these points are discussed below.

The difference in response of a tumor to pions and x-rays will be determined by the interaction between the beam characteristics (dependence on oxygen concentration for injury induction, type of injury induced, and the characteristics of the tumors, e.g., hypoxic fraction, rates of repair and recovery). Experiments relating to the maximization of pion injury to tumor tissues and to minimization of injury to normal tissues are addressed as mechanism studies.

Finally, to anticipate the unexpected, a series of tests will be developed to provide rapid yet accurate answers to questions which develop in the course of the year. The development of these values is discussed as baseline studies.

(1) Beam Development Studies

These studies are designed to provide the information required for the development of isoeffect regions in the peak of the pion beam and to establish the shape of the beam in terms of biological effectiveness.

(a) Depth Dose Distribution and Relative Biological Effectiveness

Preliminary studies using several techniques have provided reproducible data depicting the shape of the lethal biological effect of the low-dose rate (3 to 6 rads/minute) beam. Human kidney T-1 and Chinese hamster ovary (CHO) cell supported on coverslips and cells supported in a gelatin matrix both have shown the expected diminished survival in the stopping region. This basic experiment will be repeated at the higher dose rates (~ 50 rads/minute) using the coverslip technique which provides the best dimensional stability along the z axis. Both of the aforementioned cell lines will be studied since both have been used for beam characterization in other laboratories and both grow well on polycarbonate coverslips which minimize dosimetric problems. Polycarbonate is also adaptable to the experiments requiring large surface areas to be exposed (determination of beam shape and sharpness).

The experiments will be conducted in replicate with the conventional cell survival statistics being employed for analysis. With the rapid dose rates available, it will be possible to select total doses which uniformly fall on the exponential portion of the cell survival curves, thereby facilitating simple calculation of the RBE.

For the determination of the RBE, cell survival estimates will be made at 0.5 cm intervals throughout the beam path. For the definition of beam shape, initial studies will employ dense platings on large areas for general orientation, followed by single cell survival studies within the areas of dose fall-off.

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(b) Oxygen Enhancement Ratio

When the higher dose rates become available, analysis of the OER as a function of depth dose will be performed in two systems: mammalian cell survival in culture, and the 10-day Vicia faba root growth system. In both types of assay, anoxia will be achieved by use of nitrogen flow prior to and during exposure. Determination of the OER will be made at 0.5 cm intervals along the beam axis.

(2) Mechanism Studies

The following experiments have been designed to determine the optimal conditions for the use of pions in clinical therapy, i.e., the conditions under which tumor injury will be maximized and normal tissue injury will be minimized. Two variables can be altered to achieve the desired results, the dose size and the dose spacing, but the requirements of maximum tumor injury and minimum normal tissue injury dictate different approaches. As an example, large doses and short recovery intervals would be desirable to maximize tumor injury, but small doses and long recovery intervals are needed to minimize normal tissue injury. The optimal conditions will represent a compromise between these two extremes, and the questions posed by these experiments concern exploitation of the unique characteristics of pions in reaching this end. For example, can low radiation doses be used so that the normal tissues are spared, while tumor injury remains near a maximum due to the RBE and OER for pions? Further, can longer recovery intervals be employed so that normal tissues recover, while tumor tissues show the characteristic slow recovery of high-LET injury? The majority of the experiments described below deal with model systems, but from studies of individual tissue responses, it is hoped that the resultant data will be extrapolable or at least sufficient to allow design of simple experiments for the other tissues.

(a) In Vitro Recovery

With the availability of higher dose rates, it will be possible to expand the preliminary data on repair which we presented earlier and improve the methodology. Standard split-dose experiments will be performed with the T-1 and CHO cell lines with x-rays and pions under both oxygenated and anoxic conditions. The pion experiments will improve various beam path positions including the plateau, peak, and post-peak regions. In this way, we will be able to describe the extent of recovery of both euoxic and anoxic cells at varying depths in tissue and compare them to similar results for x-rays.

While the time course for the foregoing experiments will be restricted in terms of the recovery interval, additional experiments will be conducted with the T-1 cells on the time course of recovery from paired equal doses of pions (peak and plateau) and x-rays in both the sublethal and lethal ranges. In this same system, complete survival curves will be obtained six hours after 300 rads of peak pions, 500 rads of plateau pions, and 500 rads of x-rays as well as for peak and plateau pions given as a series of one, two, three, or four fractions separated by three hours.

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