Comparison of Effects of Daily versus Hyperfractionated Split-Course Radiation Schedules with and without Cyclophosphamide on Median Survival, Metastatic Dissemination, Tumor Cure, and Growth Rates


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ABSTRACT

Daily fractions of 188, 250, 375, 500, and 750 rads were given to rats with hepatoma 3924A so that all groups received the same weekly dose of 1500 rads over a 6-week period, for total doses of 9000 rads when only radiation was given and 4500 rads when combined with cyclophosphamide. No tumors were cured (with two exceptions) with or without three doses of cyclophosphamide (150 mg/kg or 0.9 g/sq m) given 14 days apart. The addition of cyclophosphamide to the daily radiation treatment schedules did not change the time for tumors to reach 8 times the volume at time of treatment but did result in a longer median survival, which was attributed to a reduction of pulmonary metastases.

A hyperfractionated radiation schedule using six 250-rad fractions given three times daily every 4 hr for 2 days combined with cyclophosphamide (150 mg/kg) 1 day later and repeated two additional times at 11-day intervals for a total dose of 4500 rads and cyclophosphamide (450 mg/kg) resulted in eradication of six of ten tumors, for a cure rate of 60%. Skin damage, determined by visually scoring the skin, appeared to be fully recovered by Day 126 and remained so until the end of the experiment on Day 384. The three courses of hyperfractionated radiation (total dose, 4500 rads), when given alone, were ineffective in producing tumor regression and cure.

Combining cyclophosphamide with hyperfractionated split-course radiation schedules gave a major increase in tumor cure rate as compared with radiation alone at the same (4500 rads) or higher (9000 rads) doses. The major gains in effective utilization of the two modalities is greatly diminished or lost when the radiation is administered as daily fractions.

INTRODUCTION

There are 5 radiation dose fractionation schedules currently under active investigation in clinical radiotherapy: (a) hyperfractionation, conventional radiation doses per fraction (150 to 300 rads) given 2 to 3 times daily; (b) superfractionation, small radiation doses per fraction (55 to 150 rads) used 2 to 4 times daily; (c) hypofractionation, higher doses per fraction given fewer than 5 times per week; (d) split-course fractionated radiation, radiation followed by a period of rest (1 to 2 weeks) between courses; and (e) intraoperative radiation, 2000 to 3000 rads given as a single dose directly to the tumor following surgical exposure (1, 3, 6, 10, 11).

The clinical importance of these experimental studies was underscored at a recent workshop on time-dose relationships in clinical radiotherapy held by the Radiotherapy Development Branch, Cancer Therapy Development Program, Division of Cancer Treatment, NIH. The evaluation of alternative time, dose, and fractionation schedules is a key element of the Branch's goal to support improvements in the results of photon and/or electron (low-linear-energy transfer) radiotherapy. Alternative fractionation schemes, different from the conventional schedule of 180 to 200 rads/day, 5 fractions/week, are being studied to determine if they can increase the probability of tumor control, decrease injury to normal tissue, optimize treatment in terms of patient convenience or economy without loss of benefit, or enhance the effectiveness of adjuvant modalities such as chemotherapy, radiosensitizers, hyperthermia, and surgery (7). Participants at the workshop concluded that conventional fractionation schemes remain the hallmark of radiotherapy and that alternative schemes must be studied carefully with prolonged periods of observation to evaluate late as well as short-term effects (such as toxicity) in terms of local tumor control and patient survival. They considered that hyperfractionation has a number of theoretical advantages, including the potential sparing of normal tissue from radiation injury. Clinical observations suggest that normal tissue tolerance may be improved, but data are unclear regarding efficacy.

Our experimental studies (8, 9) with hepatoma 3924A in rats have shown that increases in tumor cure rates can be obtained when hyperfractionated split-course radiation schedules are combined with cyclophosphamide, compared to the same radiation administered alone. Therefore, one of the primary objectives of this study was to determine if similar increases in therapeutic gain could be obtained by daily fractionated radiation combined with cyclophosphamide, as was previously obtained using hypofractionated and hyperfractionated split-course radiation with cyclophosphamide.

MATERIALS AND METHODS

Solid Tumor Line. Experimental tumors were obtained by injecting 3924A tumor cells into the backs of ACI rats. A mince, prepared from viable tissue and loaded into 1-ml syringes, was delivered s.c. into the animals through a 14-gauge needle. Direct counting of nuclei prepared by homogenization of the tumor in citric acid and stained with crystal violet gives approximately $2.5 \times 10^6$ cells/g, of which approximately 20% are diploid host cells and 80% are hypotetraploid 3924A cells in G1, S, or G2-M (by flow microfluorometric analysis). Therefore, delivery
of 0.1 ml of tumor mince corresponds to 20 x 10^6 tumor cells. Implants using 0.05 or 0.02 ml of mince are more variable in the time to achieve 42 hr; and cell cycle time, 27.4 hr. The different cycle phases are: actual volume doubling time, 104 hr; potential volume-doubling time, over the past 10 years have shown hepatoma 3924A to be stable and reproducible. The kinetics of cell proliferation and tumor growth are: Td, 14 hr; Ts, 9.3 hr; To, 3.7 hr; and Tu, 0.4 hr. The 1-hr thymidine labeling index is 17.6, the growth fraction is 0.65, and the cell loss factor is 0.60 (5).

Some treatment protocols reduce growth of the primary tumor, but lung metastases develop after 60 days. Cytogenetic analysis has established that these metastases are from the primary tumor. Since only 25 doublings occur between one cell and a 0.2-g tumor, the time of appearance of these metastases is consistent with growth from single cells, which indicates low immunogenicity for this tumor. Also, tumors grew at rates comparable to those in animals growing their first tumor when 3 groups of animals which had been cured for average periods of 20, 107, and 282 days were reinoculated with 3924A. Any immune response which occurred as a result of the first tumor had little effect on subsequent growth or "tumor takes" of reinoculated 3924A cells.

Tumor Radiation. Local tumor radiation was carried out with a 250-kV 15-ma GE Maximar 250-III, with a half-value layer of 1.39 mm Cu. Radiation was delivered at a rate of 280 rads/min and was filtered through 0.5 mm Cu and 1.0 mm Al. Before radiation, the animals were anesthetized with ether and placed in a lead shield through which the tumor protruded. The tumor received the dosages indicated, while the rest of the animal received approximately 0.5% of that dose.

Cyclophosphamide. Cyclophosphamide was supplied by Mead Johnson Research Center, Evansville, Ind. It was dissolved in 0.9% NaCl solution and given by i.p. injection. Each dose was given as a single injection.

Radiation Damage to Normal Tissue. A method of scoring skin reaction to different radiation doses has been developed by Fowler et al. (5). Their procedure has been modified slightly for this experiment, since we irradiated the skin on the tumor over the abdominal flank of the rat, whereas Fowler irradiated the foot of the mouse. Scoring of skin reaction in these experiments is: 0.0, no detectable damage; 0.5, discoloration but no dryness; 1.0, slightly scaly; 1.5, scaly appearance; 2.0, dry scab; and 2.5, moist scab.

Tumor Volume Measurements and Tumor Growth Analyses. The growth rate of hepatoma 3924A was found through a combination of frequent tumor volume measurements and computer methods of data handling. Tumor volumes were determined from vernier caliper measurements of length (L), width (W) and height (H) made daily for 1 to 2 weeks after treatment, during the period of major changes in growth rates. Tumors were then measured 3 times weekly until termination of the experiments. Tumor volume is well approximated using a hemiellipsoid in which volume = 4π/3 x L/2 x W/2 x H/2, which reduces to one-half of the product of the length of the 3 axes, i.e., V = ½ L WH. Tumors were initiated when animals could be grouped with a mean tumor volume of 200 to 300 cu mm. Tumor volumes (10 animals/group) were averaged for each treatment or control group for presentation as growth curves. The time required for individual tumors to reach a volume of 8 times the treatment volume (8V0) was determined by interpolation between measurements, and these values were averaged for presentation in tabular form or used individually in the Cox model discussed below.

For growth rate determinations, tumor volumes for each animal are normalized and log-scaled to produce a series of curves with

\[ \ln \frac{V(t)}{V_0} \]

versus time, in which V(t) is the volume at time t and V0 is the volume at the beginning of treatment. Local error may then be reduced by smoothing each of the curves with a variable point-averaging filter in which a moving average of each point and a number of its neighbors (usually one on each side) is taken in both fractional volume and time. The derivative of these smoothed curves are obtained via a standard, discrete, central difference method of the form:

\[ GR(T) = \frac{V_j + 1 - V_{j - 1}}{\tau_j + 1 - \tau_{j - 1}} \]

where j is a measurement point within the experimental period corresponding to a smoothed, log-scaled, fractional volume, V, \( \tau \) is the adjusted time, and GR is the growth rate associated with time T, taken midway between \( \tau_j \) and \( \tau_{j - 1} \). The individual central difference curves, i.e., growth rates for each of the animals, are ensemble averaged to provide a mean growth curve unique for the treatment protocol used within the group.

The relationship between survival time and number of rads per fraction was modeled using Cox’s proportional hazards model (4) which was linear in \( \chi \), the number of rads per fraction, i.e.,

\[ \lambda(t) = \lambda_0(t) \exp(\beta_1 X_1) \]

Here, \( \lambda(t) \) is the risk of death at time t associated with a given number of rads per fraction, \( \chi \); \( \lambda_0(t) \) is the hazard or risk associated with no radiation, and \( \exp(\beta_1 X_1) \) is the proportionality constant. [It should be noted that knowledge of \( \lambda_0(t) \) is not required in this approach.] In this formulation, \( \beta_1 \) parameterizes the model. The method of maximum likelihood was used to estimate the model parameter. Often treatment toxicity will result in a curvilinear relationship between survival time and treatment levels. When this is the case, a second-degree polynomial model has been shown to be useful (2, 8). However, for the range of rads per fraction used in this study, the addition of a second-degree term did not result in a significant increase in the likelihood of the sample. For this reason, the linear model was used. Similar considerations led to the use of a first-degree polynomial in Cox’s proportional hazards model to approximate the relationship between time to BV0 and number of rads per fraction. Where deaths occurred before BV0 was attained, the data for these animals were treated asensored observations.

This model was chosen because it provides a method for relating treatment levels to survival times. Under “Appendix,” we give the results of several different ways of assessing the adequacy of the model. Since there was no indication that the model was inappropriate, we chose to use it to describe the rads/fraction-survival (dose-response) relationship. The model does not speak to the mechanism of action for the treatment. Thus, it is not encumbered by any restrictive assumptions concerning how the treatment prolongs survival.

RESULTS

Daily Radiation Fractionation Schedules with and without Cyclophosphamide

The protocol for daily radiation fractionation schedules with and without cyclophosphamide is given in Table 1. The groups given radiation alone received 1500 rads/week over a 6-week period. For daily radiation combined with cyclophosphamide, the 2 modalities were alternated so that radiation (1500 rads) was given during Weeks 1, 3, and 5 and cyclophosphamide (150 mg/kg) was given once weekly at the beginning of Weeks 2, 4, and 6.

Relationship between Survival Time and Number of Rads per Fraction with and without Cyclophosphamide. Median survival decreased from 143 days for the group given 500

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rads/fraction to 130 days for the 188-rads/fraction group. Median survival time for radiation combined with cyclophosphamide decreased from 232 days for the 750-rads/fraction group to 122 days for the 188-rads/fraction group (Table 2). The survival data were modeled using Cox’s hazards model (4), and these results are presented under “Appendix.” For both radiation alone and radiation plus cyclophosphamide, decreasing the number of rads per fraction was associated with decreased survival time for the range of rads per fraction considered.

Relationship between Tumor Growth and Number of Rads per Fraction Given Daily with and without Cyclophosphamide. The times for tumor volumes to reach 8 times initial volume (8V₀) decreased from 121 ± 7.5 (S.E.) days for the 500-rads/fraction-group to 91 ± 3.4 days for the 188-rads/fraction group for radiation alone (Table 2). Control tumors reached 8V₀ in 9.27 ± 0.76 days, which was so short a time (≤ 10%) compared to that for treated tumors that it was not subtracted from the treated groups to obtain classical growth delay values. The time to reach 8V₀ in the groups given radiation combined with cyclophosphamide decreased from 147 ± 6.3 days for the 750-rads/fraction group to 85 ± 2.9 days for the 188-rads/fraction group (Table 2). Using Cox’s model (see “Appendix”), a significant relationship between number of rads per fraction and time to 8V₀ was found both for radiation alone and for the combined therapy; as the number of rads per fraction increased, time to 8V₀ increased.

Daily irradiation with 250 rads/day, 6 days/week, to a total dose of 9000 rads prevented significant growth of hepatoma 3924A during the treatment period (Chart 1), but only one cure was obtained. The nadir for tumor reduction occurred 42 days after initiation of treatment and 2 days after termination of treatment on Day 40. Mean tumor volume was 202 ± 49 cu mm, similar to initial mean tumor volume of 227 ± 13 cu mm before beginning treatment. There was a gradual rise in mean tumor volume after termination of treatment; time to reach 8V₀ was 108 ± 51 days (Table 2).

When radiation was given as 6 daily 250-rad fractions alternated with cyclophosphamide at 7-day intervals for 3 courses, there were no complete tumor responses and no tumor cures (Chart 2). The nadir for tumor volume reduction of 206 ± 23 cu mm occurred on Day 45, which was comparable to mean tumor volume of 243 ± 8 cu mm at the beginning of treatment. There was a gradual increase in tumor volume over the 2

Table 1
Protocol for different daily rads per fraction with and without cyclophosphamide

<table>
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<tr>
<th>Group</th>
<th>No. of fractions</th>
<th>No. of rads/fraction</th>
<th>Dose/ course (rads)</th>
<th>Total dose (rads)</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
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</table>

a Ten rats/group.
b Subsequent radiation courses were initiated on Days 7, 14, 21, 28, and 35. Subsequent combined radiation-cyclophosphamide courses were initiated on Days 14 and 28.
c Repeated daily through Day 47.
d CP, cyclophosphamide, 150 mg/kg on Days 7, 21, and 35.

Table 2
Tumor growth delay, median host survival, and incidence of pulmonary metastases for different daily rads per fraction with and without cyclophosphamide

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of fractions/ course</th>
<th>No. of rads/ fraction</th>
<th>Total radiation dose (rads)</th>
<th>Time (days) to reach 8V₀</th>
<th>Median survival time (days)</th>
<th>Incidence of pulmonary metastases (%)</th>
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<td>Radiation</td>
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<td>90</td>
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<tr>
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<td>375</td>
<td>9000</td>
<td>105 ± 6.0</td>
<td>134</td>
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<tr>
<td>D</td>
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<td>250</td>
<td>9000</td>
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<td>90</td>
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<td>188</td>
<td>9000</td>
<td>91 ± 3.4</td>
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<td>100</td>
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<tr>
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<td>750</td>
<td>4500</td>
<td>147 ± 6.3</td>
<td>232</td>
<td>50</td>
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<tr>
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<td>3</td>
<td>500</td>
<td>4500</td>
<td>141 ± 7.2</td>
<td>170</td>
<td>60</td>
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<td>G</td>
<td>4</td>
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<td>98 ± 5.6</td>
<td>125</td>
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<td>4500</td>
<td>87 ± 2.5</td>
<td>122</td>
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<td>C</td>
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<td>188</td>
<td>4500</td>
<td>85 ± 2.9</td>
<td>123</td>
<td>80</td>
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</table>

a Ten rats/group.
b 8V₀, time to reach 8 times the volume at initiation of treatment.
c Median survival time for controls was 40 days.
d Incidence of pulmonary metastases in controls was 10%.

* Mean ± S.E.
months following termination of treatment, which reached 1212 ± 369 cu mm on Day 98. The time to reach $8V_0$ was 87 ± 2.5 days, and median survival for this group was 122 days (Table 2).

**Hyperfractionated Radiation Schedules with and without Cyclophosphamide**

A hyperfractionated radiation schedule using six 250-rad fractions given at 8 a.m., 12 noon, and 4 p.m. on Days 0 and 1 and repeated at 7-day intervals to a total of 9000 rads produced no tumor cures (Table 3, EXP 775-H). The nadir for volume reduction was reached 45 days after initiation of treatment and 9 days after termination of treatment on Day 36. Mean tumor volume on Day 45 was 85 ± 46 cu mm, less than one-third of the initial volume of 267 ± 16 cu mm (Chart 3), and it gradually increased to 1649 ± 1209 cu mm by Day 98. Time to reach $8V_0$ was 147 ± 13 days.

Results of another hyperfractionated split-course radiation experiment are summarized in Table 3 and shown in Chart 4A. Three 250-rad fractions were given at 8 a.m., 12 noon, and 4 p.m. on Days 0 and 1 as in the preceding hyperfractionated split-course experiment; however, these courses were repeated at 11-day intervals as opposed to intervals of 7 days in the previous experiment. Total radiation was one-half of that given previously (4500 versus 9000 rads); no tumors were cured.

When the time interval between hyperfractionated schedules was lengthened from 7 to 11 days, radiation alone was not adequate for control of tumor growth during the treatment period (Chart 4A). The time to reach $8V_0$ was 38.2 ± 3.48 days, and median survival was 73 days (Table 3, EXP 774-E). Growth rates determined by central difference, as detailed in "Materials and Methods," were reduced compared to control rates but did not become negative during treatment when the time between hyperfractionated split-course schedules was 11 days (Chart 4B). Growth rates remained depressed for 49 days between termination of treatment on Day 23 and the end of accurate determinations on Day 72.

Results of the 250 rads/fraction combined with cyclophosphamide (summarized in Table 3) demonstrate the increased effectiveness of hyperfractionated radiation schedules interacted with cyclophosphamide as compared with the same hyperfractionated schedules alone. The addition of cyclophosphamide 1 day after each hyperfractionated course given as 250-rad fractions at 8 a.m., 12 noon, and 4 p.m. on Days 0 and 1 resulted in a continuous, rapid decline of mean tumor
Table 3

Effect of hyperfractionated radiation schedules with and without cyclophosphamide on tumor cure rates and median survival

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<th>EXP 775</th>
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<tr>
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<td>12</td>
</tr>
<tr>
<td></td>
<td>0 1 11 11 22 22</td>
<td>14 14 14 14 14 14 14 14</td>
</tr>
</tbody>
</table>

* CP, cyclophosphamide; total dose was given as three 150-mg/kg doses at 11-day intervals.
<sup>c</sup> 8V0, time to reach 8 times the volume at initiation of treatment. The time for controls in EXP 774 to reach 8V0 was 4.93 ± 0.13 days. Time to 8V0 in EXP 775 was 5.95 ± 0.29 days.

Chart 3. Mean tumor volumes after hyperfractionated radiation (R) at 7-day intervals (250 rads/fraction at 8 a.m., 12 noon, and 4 p.m. on Days 0 and 1, repeated weekly to a total dose of 9000 rads). O, treated; •, control; bars, S.E.

Chart 4. A, mean tumor volumes after hyperfractionated radiation (R) at 11-day intervals (250 rads/fraction at 8 a.m., 12 noon, and 4 p.m. on Days 0, 1, 11, 12, 22, and 23, to a total dose of 4500 rads). B, mean growth rate for tumors in A given hyperfractionated radiation (R) at 11-day intervals. Bars, S.E.
Radiation Schedules Alone and with Cyclophosphamide

Chart 5. A, mean tumor volumes after combined cyclophosphamide (cp) and hyperfractionated radiation (R) at 11-day intervals (250 rads/fraction at 8 a.m., 12 noon, and 4 p.m. on Days 0, 1, 11, 12, 22, and 23, to a total dose of 4500 rads; cyclophosphamide, 150 mg/kg on Days 2, 13, and 24). B, mean growth rate for tumors in A given combined cyclophosphamide (cp) and hyperfractionated radiation (R) at 11-day intervals. Bars, S.E.

volumes during and after the 23-day period of treatment (Chart 5A). Combined use of chemotherapy and radiotherapy given as split courses at 11-day intervals resulted in complete response in 6 of 10 tumors 54 ± 7 days after initiation of treatment, for a cure rate of 60%, since no tumors regrew. Partial response occurred (≥50% reduction in volume) in the remaining tumors (Table 3, EXP 774-H). Median survival was greater than 384 days at termination of the experiment on Day 384.

The growth rate rapidly became negative during the first week of treatment when cyclophosphamide was administered with each split course of hyperfractionated radiation (Chart 5B), which is in contrast to the split-course hyperfractionated radiation given alone (Chart 4B). The magnitude and duration of negative growth was also greater and longer than in the experiment with daily fractionated radiation plus cyclophosphamide (data not shown). The increased effectiveness of combined-modality split-course hyperfractionated therapy is further demonstrated by the fact that a cure rate of 60% was obtained, contrasted with no cures when the same total dose of radiation was given in daily fractions with cyclophosphamide.

Pulmonary Metastases after Radiation with and without Cyclophosphamide

The incidence of pulmonary metastases in animals given radiation alone was 90 to 100% (Table 2), which contrasts with an incidence of 10% in controls. There was a constant increase in pulmonary metastases from 50% for the 750-rads/fraction group to 80% for the 188-rads/fraction group with cyclophosphamide.

Normal Tissue Reaction to Radiation

The effects of radiation on skin were determined by using the methods of Fowler et al. (5). Skin reaction was well below their acceptable limit of 1.5. Maximum acute skin reaction for the group given 9000 rads as daily 250-rad fractions (tumor and survival response is given in Table 2, Group D; Chart 1) occurred 45 days after initiation of treatment. Mean reaction was 1.15. For the group given 9000 rads as hyperfractionated split-course schedules on a 7-day cycle (Table 3, EXP 775-H; Chart 3), maximum acute skin reaction occurred 49 days after initiation of treatment and was 1.00. In the group given 4500 rads as 250-rad fractions daily on alternate weeks with cyclophosphamide (Table 2, Group E; Chart 2), maximum reaction occurred 45 days after initiation of treatment. Mean reaction was 0.55. Maximum acute skin reaction for the group given 4500 rads as 250-rad hyperfractionated split courses with cyclophosphamide on an 11-day cycle (Table 3, EXP 774-H; Chart 5A) occurred on Day 28; mean reaction was 0.55. Skin reaction remained elevated for 70 to 84 days and then gradually returned to normal 126 days after initiation of treatment. No evidence of chronic skin damage was observed between Day 126 and termination of the experiment on Day 384.

DISCUSSION

Hyperfractionated split-course radiation schedules were previously carried out (8, 9) using the same number of rads per fraction as used here for the daily fractionation. The major difference in scheduling was that 1500-, 750-, 500-, 375-, 250-, and 188-rad fractions were given over a 2-day period and repeated every 11 days for 3 courses. The three 1500-rad courses were given on Days 0 and 1, 11 and 12, and 22 and 23, for a total dose of 4500 rads, which is in contrast to the current experiment in which daily fractions were given in 6 weekly 1500-rad courses, for a total of 9000 rads.

The tumor cure rate for the hyperfractionated radiation schedules in the previous study was 40, 10, 0, and 0% for the 1500-, 750-, 500-, 375-, 250-, and 188-rad fractions, respectively. In the present study, in which twice the total radiation dose was given, but as daily fractions, one animal of 10 in the 500- and 250-rads/fraction group had its tumors cured, and no cures were present in the 375- and 188-rads/fraction groups. Results from both studies indicate that the higher-rad/fraction schedules were more effective in controlling tumor growth, producing...
tumor cures, and increasing life span.

A marked increase occurred in tumor cure rates in the hyperfractionated split-course study (8, 9) when cyclophosphamide (150 mg/kg) was given 1 day after each of the radiation courses on Days 2, 13, and 24. Cure rates were 80, 80, 70, 60, and 50%, respectively, for the 750-, 500-, 375-, 250-, and 188-rads/fraction groups. In contrast, no tumor cures occurred in the 500-, 375-, 250-, and 188-rads/fraction groups, and only one tumor of 10 was cured in the 750-rads/fraction groups when radiation was given daily in the current study. The same total radiation (4500 rads) and cyclophosphamide (450 mg/kg) doses were given in both experiments.

The incidence of pulmonary metastases increased as the number of rads per fraction decreased in the previous hyperfractionated split-course study, being 10, 80, 90, and 90% for the 1500-, 750-, 500-, and 250-rads/fraction groups given radiation alone. The addition of cyclophosphamide 1 day after the end of each of the 3 radiation courses eliminated pulmonary metastases in all groups. All but 2 animals given radiation alone developed pulmonary metastases in this study using daily fractions, even though the radiation dose was 9000 rads, compared to 4500 rads in the previous experiment (Table 2). The addition of cyclophosphamide at the end of each of the 3 radiation courses given as daily fractions was less effective in controlling metastatic dissemination than was cyclophosphamide given with hyperfractionated radiation in the previous study. The incidence of pulmonary metastases increased from 50% in the 750-rads/fraction group to 80% for the 188-rads/fraction group (Table 2).

The administration of cyclophosphamide in this and the previous study reduced the incidence of pulmonary metastases. The increase in median survival times in the groups given cyclophosphamide in this study could therefore be a result of reducing metastatic dissemination, since no apparent difference occurred in the ability to control primary tumor in groups with and without cyclophosphamide.

Our studies have shown that acute skin reaction is reduced as the size of the fraction is reduced and that administering cyclophosphamide 24 hr after radiation has no apparent effect on skin (8, 9). There was a linear reduction in maximum skin reaction from 1.94 for the 1500-rads/fraction group to 0.50 for the 188-rads/fraction group on Day 42. The coefficient of determination was 0.93, with a slope of 0.00103 per rad. Steel et al. (12) also have found no increase in average skin reaction when cyclophosphamide is combined with radiation, compared with radiation alone. Skin of animals given the highest dose per fraction (1500 rads) did not begin to recover until Day 161; however, for the 2 lesser doses (250 and 188 rads), there were no detectable radiation effects beginning on Day 126 and continuing until Day 384. These results suggest that skin appears to have fully recovered after multiple radiation doses over a 1- to 2-day period when the numbers of rads per fraction are similar to those extensively used in clinical radiotherapy. However, radiobiological recovery, which will require evaluation after retreatment of the irradiated area, has not yet been demonstrated for these radiation treatment schedules.

A review article by Withers et al. (13) concluded that the late effects of radiation injury in normal tissues are most probably the result of division-cycle-related death of slowly dividing parenchymal and/or stromal cells. This concept simplifies the understanding of dose-response relationships and suggests directions for research in reducing late effects. The authors state that, if late injury in normal tissue is not the result of vascular injury but of death of slowly proliferating cells, slow regeneration of survivors is possible, and long-established policies regarding retreatment of previously irradiated areas may deserve review.

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APPENDIX

Analysis of Median Survival and Tumor Growth Results

**Median Survival.** For survival after radiation alone, the maximum likelihood estimate in Cox’s model for \( \beta_1 \) was -0.00056. There is a significant \( (p = 0.0374) \) correlation (Spearman’s) between estimated in [x/0] and median survival time. While this does not necessarily indicate model fit, it does suggest that there is a statistically significant relationship between observed and modeled data. Quadratic models did not significantly improve the fits; therefore, we chose to stay with a first-degree polynomial model. The \( p \) value associated with the test \( H_0: \beta_1 = 0 \) was 0.0001. Therefore, we conclude that \( \beta_1 \) was significantly different from zero. Since the algebraic sign is negative, we have an indication that, for the range of rads per fraction considered, increasing the number of rads per fraction is associated with increased survival time.

For combined therapy, \( \beta_1 \) from Cox’s model was estimated to be -0.0056 with \( p < 0.0001 \), indicating again a significant association between survival and increasing rads per fraction. Again, quadratic models did not significantly improve the fits.

The log rank test was used to determine if a difference occurred in median survival times for groups given the same number of rads per fraction with and without cyclophosphamide. The \( p \) values for the log rank test were 0.05, 0.42, 0.01, and 0.11 for the 500-, 375-, 250-, and 188-rads/fraction groups, respectively. There appeared to be a difference in survival distribution in groups given cyclophosphamide and radiation compared to groups given radiation alone, with the exception of the 375-rads/fraction group.

**Tumor Growth.** For time to 8Vo after radiation alone, the maximum likelihood estimate in Cox’s model for \( \beta_1 \) was -0.0079. Spearman’s rank correlation coefficient is significant and indicates that the modeled data and the observed data are related. Again, there was no improvement by considering a quadratic model. The \( p \) value associated with the test \( H_0: \beta_1 = 0 \) was <0.0001. There was a significant relationship between number of rads per fraction and time to 8Vo. Since the algebraic sign is negative, we can conclude that as numbers of rads per fraction increase, time to 8Vo will increase.

The time to reach 8Vo in the groups given radiation combined with cyclophosphamide decreased from 147 ± 6.3 days for the 750-rads/fraction group to 86 days for the 188-rads/fraction group. The maximum likelihood estimate in Cox’s model for \( \beta_1 \) was -0.0068. The \( p \) value (<0.0001) associated with Spearman’s correlation is significant and indicates that the modeled data and observed data are related. The \( p \) value associated with the test \( H_0: \beta_1 = 0 \) was <0.001; thus, there is a significant relationship between the number of rads per fraction and the time to reach 8Vo. There was a linear increase in the time to reach 8Vo as the number of rads per fraction increased.

The log rank test was used to determine if a difference occurred in the time to reach 8Vo for groups given the same number of rads per fraction with and without cyclophosphamide. The \( p \) values for the log rank test were 0.40, 0.27, 0.18, and 0.29, respectively, for the 500-, 375-, 250-, and 188-rads/fraction groups. No apparent difference in distributions of time to reach 8Vo was present in the group given cyclophosphamide and radiation as compared to the group given radiation alone.

REFERENCES


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