The Influence of ICRF-159 and Levamisole on the Incidence of Metastases Following Local Irradiation of a Solid Tumor

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Courses of irradiation consisting of 6000 rad in ten equal fractions over 12 days delivered to KHT sarcomas in mice controlled 55% of the local tumors but 83% of the mice died from metastases. Three strategies to reduce the risk of metastatic spread were tested. The fractionation scheme was changed to deliver the same total dose using a large initial fraction followed by seven equal portions with the same overall time. ICRF-159 was used with the intention of partially synchronizing the tumor growth fraction in a radiosensitive state of the growth cycle and of promoting normalization of the tumor vasculature. Levamisole was used to stimulate the immune system. The combination of ICRF-159 with the eight-fraction radiation course proved to be effective for both increasing local control and decreasing the incidence of metastases. The addition of levamisole did not improve the results obtained with a combination of ICRF-159 and irradiation.

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DATA FROM animal model systems have indicated that local irradiation of solid tumors may increase the incidence of metastatic disease.[1-4] Studies leading to a contrary conclusion have been less frequent.[5] Results of some clinical investigations have also suggested that although radiation therapy is effective in local tumor control, it may increase the risk of distant metastases.[6-9] Other investigators have concluded that irradiation has no effect[10] or that it may reduce the risk of distant metastases.[11] Results of studies from this laboratory have shown that when a KHT sarcoma in the leg of a C3H/He mouse is irradiated to an x-ray dose that will locally control 50% of the tumor, the incidence of metastases is higher than that found in the nontreated mice.

A variety of mechanisms by which x-irradiation might influence metastatic spread have been proposed.[12,13] A combination of at least three processes seems to be implicated: (1) the interaction of frequent tumor cell detachment and inherent mobility which are common properties of malignant neoplasms together with the increased tissue permeability that is a universal response to x-radiation; (2) the sequestering of disseminated tumor cells in microthrombi; and (3) the supression of cellular immune mechanisms which may develop following irradiation.

The present study was undertaken in order to compare the results of three therapeutic strategies intended to use the effectiveness of irradiation to achieve local control and to reduce the incidence of metastases. The first strategy was to make the initial fraction of radiation large so as to inactivate clonogenic cells that might be disseminated during the course of irradiation.

The second strategy was to use daily injections of ICRF-159, (± 1,2-bis (3 5-dioxopiperazin-yl) propanel) (Drug Synthesis and Chemistry Branch DTP, TCT, NIH, Bethesda, MD). This drug blocks progression through the cell cycle in late G_2 or G_2/M where clonogenic cells would be most radiosensitive.[14,15] At drug concentrations that produce negligible systemic toxicity, there is evidence that ICRF-159 normalizes the development of tumor vasculature.[16-19] These effects would be expected to reduce both the total number of clonogenic cells available for dissemination and the number that might escape from the irradiation volume.

The third strategy was to stimulate the host immune system. For this purpose, the nonspecific immune stimulant levamisole (Janssen R and D, Inc., New Brunswick, NJ) was used.[20,21] Levamisole was selected on the basis that it would restore or minimize any immune suppression induced by the ICRF-159 or the radiation.[21,22]

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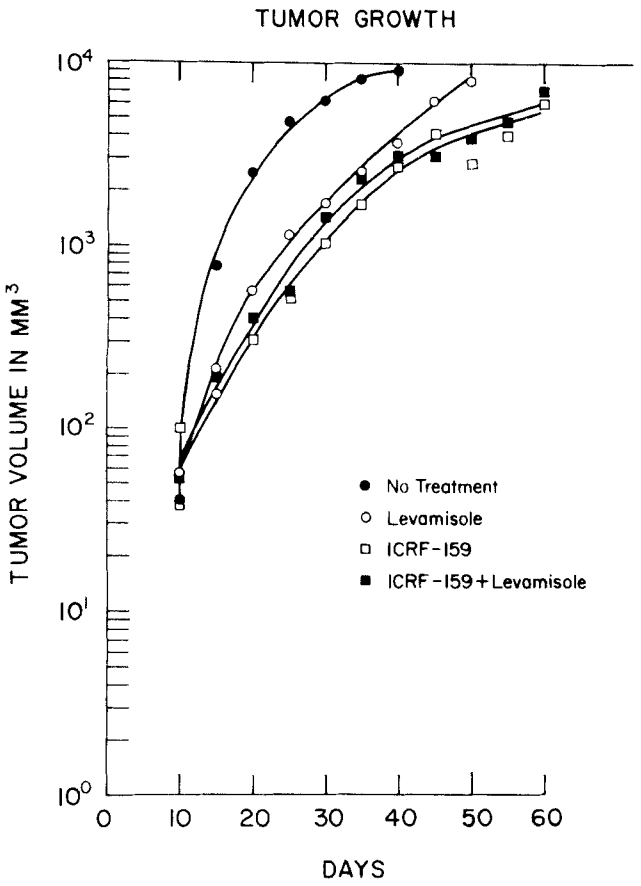

FIG. 1. Growth of tumors in untreated and drug-treated mice.

In this report, we describe the effects of these strategies on the responses of the primary tumors and on the incidence and sites of metastases.

Materials and Methods

Animals

Female C/3H/He mice (Jackson Laboratories, Bar Harbor, MN) were used. Animals were 75 to 100 days old at the start of the experiment.

Tumor

The tumor model was the KHT sarcoma which originally arose in the ear of an untreated mouse.[23] This tumor has been maintained by serial passage using tumor fragments in C3H/He mice in this laboratory since 1967. Tumor passages 320–340 were used in this study. In each animal, a 1-mm^3 tumor fragment was implanted by trochar into the subcutaneous tissue of the lateral aspect of the right thigh. The tumor becomes palpable after five to seven days. At this time the mice were randomly assigned to the various treatment groups. Tumors were measured daily in three dimensions: length (l), thickness (t), and width (w), using a micrometer and the volume V calculated as

$$V = \frac{\pi}{6} l \cdot t \cdot w.$$

Tumor growth rates and doubling times were derived from these measurements.

Irradiation

Irradiation was delivered using a 250 kVP x-ray machine filtered to a HVL of 1.4 mm^3 at a dose rate of 270 rad per minute. The dose of 6000 rad in ten equal fractions over 12 days was selected since preliminary data indicated that this treatment regiment would result in a primary tumor control rate of 50–60%. The 1800-rad fraction was used in order to determine whether a large initial fraction would reduce the risk of metastatic spread as compared with the same total dose but given in ten equal fractions. For irradiation, the mice were anesthetized with intraperitoneally administered Phenobarbital, 35 mg/kg body weight. Each was placed under a lead shield so that only the tumor-bearing leg was irradiated.[24] Regardless of group, irradiation was begun on day 10 after tumor implant.

Drug Treatments

Levamisole was subcutaneously administered at a dose of 5 mg/kg body weight every second day starting on day 9 after implant and ending on day 21. The ICRF-159 was prepared immediately before use as described by Le Serve and Hellmann.[17] It was administered by means of intraperitoneal injection at a dose of 30 mg/kg body weight daily over the same interval used for the levamisole. This was to insure the presence of the drugs during the radiation course.

Criteria of Response

Each mouse was followed until death or until it had been without tumor for 100 days. Each mouse was autopsied at death. Organs were examined for evidence of tumor metastases. In addition to the incidence and distribution of metastases, the following were noted: (1) incidence of tumor control; (2) tumor growth rate; and (3) survival time. The survival rate for mice with tumor(s) was plotted on a probit scale in order to transform the survival curves into straight lines.

There were three radiation treatment series, each with four different drug treatment groups. Series A was designed to determine the incidence of metastases in the absence of radiation. Group 1 received no treatment and Groups 2–4 received either ICRF-159 or levamisole, or both.

TABLE 1. Incidence and Sites of Metastases and Survival Times for Untreated and Drug Treated mice: Series A

	Treatment no radiation			
	No treatment	ICRF-159	Levamisole	ICRF-159 + levamisole
Tumor				
Number	21	20	22	17
Metastases	14 (67%)	17 (85%)	21 (95%)	12 (71%)
Metastatic site				
Inguinal Node	12	4	18	3
Lung	14	5	11	3
Mediastinum and chest wall	2	3	5	1
Axilla	5	14	10	8
Lumbar node	2	6	1	3
Ovary	2	1	2	1
Other sites	0	3	2	2
Average survival time (days)				
With metastases	32 (r = 20-40)	52 (r = 41-60)	40 (r = 25-55)	51 (r = 26-69)
Without metastases	29 (r = 24-36)	43 (r = 41-45)	35*	47 (r = 45-50)

* Single animal.

Series B was designed to determine the effect of ICRF-159 and Levamisole on the incidence of metastases when the tumor was irradiated with 6000 rad delivered in ten equal fractions over 12 days. Group 1 showed the incidence of metastases with radiation only. Groups 2-4 showed the effects of treatment with ICRF-159, levamisole or a combination of the drugs with the ten fraction radiation dose.

Series C was designed to determine whether modifying the uniform fraction dose schedule so that a large dose of radiation was delivered at the start of the radiation treatment would inactivate enough clonogenic cells to reduce the incidence of metastases. Group 1 showed the effect of the radiation alone. Groups 2-4 showed the effect of ICRF-159, levamisole, or a combination of the drugs with the eight fraction radiation dose.

Results

Series A

Ten days after the tumor fragments were implanted, the KHT sarcomas were in exponential growth at rates similar to those which others have reported for this tumor model[25] (Fig. 1). With no treatment, the tumors grew to an average volume of between 5 and 6 cm^3, killing the mice within approximately 30 days after the original implant (Table 1). The incidence of metastases in these mice was 67% with lungs and right inguinal nodes always involved (Table 1). Treatment with levamisole alone resulted in a decreased mortality rate (Fig. 2) as compared with no treatment. The lungs were less frequently involved in the drug-treated than in the nontreated mice (Table 1).

Series B

Following ten fractions (6000 rad) of irradiation, (Table 2) 20 of 22 tumors regressed completely and two were persistent. Of the 20 that regressed, eight recurred by 45 ± 12 days. Of the remaining 12 mice, ten died with extensive metastases by 39 ± 4 days without primary tumors and two mice survived for over 100 days with no tumors, *i.e.*, total control. When ICRF-159 was added to the radiation, the incidence of metastases was reduced as compared with x-ray treatment only. The fewer recurrences resulted in 80% incidence of total tumor control. Treatment with levamisole, however, resulted in only a small decrease in the incidence of metastases which, combined with the increased incidence of local tumor control, resulted in a 27% total tumor control as compared with only 9% for irradiation only. This was not statistically significant. Combining ICRF-159 and levamisole with the ten fraction radiation course failed to improve on the effect of ICRF-159.

Series C

When ICRF-159 was used in the eight fraction radiation course, only one of 18 mice (6%) had metastases and this animal also had a persistent tumor (Table 2). There was a 94% total tumor control rate. In the levamisole-treated group, the effect of fewer metastases and more local tumor control also resulted in an increased total tumor control rate. The addition of ICRF-159 and levamisole to the eight-fraction radiation course did not improve the results achieved with ICRF-159.

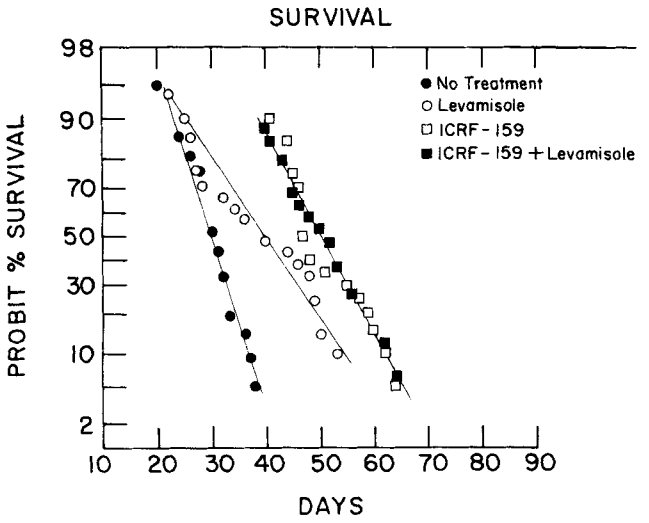

FIG. 2. Survival times for nontreated and drug-treated mice, Series A.

Although combining levamisole with the radiation treatment was more effective in reducing metastases than radiation alone, it was less effective than ICRF-159. Immune stimulation with levamisole did not potentiate the antimetastatic action of ICRF-159. Neither drug caused a consistent change in which anatomic sites metastases developed.

Discussion

In the absence of radiation, tumor growth rate was reduced to the same extent by the ICRF-159 and levamisole (Fig. 1). The effect of the ICRF-159 was probably the result of cytotoxic activity, for although the extent of tumor growth inhibition of ICRF-159 and levamisole did not differ, the survival curve for the mice receiving ICRF-159 was displaced towards a longer survival time without a significant change in the mortality rate as compared with the nontreated mice. This we interpret as indicating a cytotoxic action of ICRF-159 on clonogenic cells of tumor. The lower incidence of lung metastases in the mice given only ICRF-159 (Table 1) may indicate a lower risk of vascular spread as compared with lymphatic spread. When combined with radiation, the incidence of metastases was too small to indicate whether ICRF-159 or levamisole influenced the anatomic sites of metastases. The tumor growth inhibition with levamisole was reflected by a decrease in the slope of the survival curve without displacement towards a longer survival time (Fig. 2). Since levamisole has no reported cytotoxicity at the concentrations used,[21] immune factors may have been involved. This response was more consistent with levamisole acting to increase the rate of cell death and removal from the tumor volume,[26,27] possibly mediated through the stimulation of macrophage activity by the levamisole.[21,28-30]

When ICRF-159 was combined with the ten fraction radiation course, the significant effects were the absence of recurrence and the decrease in the number of metastases as compared with radiation alone. In a clinical trial of concomitant treatment with ICRF-159 and ir-

TABLE 2. Incidence of Metastases, Tumor Control, and Survival Times for Mice when Local Tumor was Irradiated

	Treatment			
	Radiation only	ICRF-159	Levamisole	ICRF-159 + levamisole
Series B: 10 fractions				
Tumor				
Number	22	15	22	18
Local control	12 (55%)	13 (87%)	16 (73%)	14 (78%)
Total tumor control	2 (9%)	12 (80%)	6 (27%)	14 (78%)
Metastases	19 (86%)	3 (20%)	15 (68%)	3 (17%)
Average survival time (days)				
With metastases	38 (r = 30-60)	85 (r = 67-95)	66 (r = 54-134)	127 (r = 118-133)
Without metastases	60*	—	—	90*
Series C: 6000 rad in 8 fraction with initial 1800 rad fraction				
Tumor				
Number	21	18	22	20
Local control	9 (43%)	17 (94%)	16 (73%)	17 (85%)
Total tumor control	3 (14%)	17 (94%)	9 (41%)	15 (75%)
Metastases	16 (76%)	1 (6%)	13 (59%)	5 (25%)
Average survival time (days)				
With metastases	50 (r = 30-110)	90*	65 (r = 43-112)	90 (r = 59-115)
Without metastases	35*	—	—	—

* Single animal.

radiation, the recurrence rate of soft tissue sarcomas was reduced.[31] In the present study, levamisole was less effective than ICRF-159. The combination of both drugs with radiation resulted in no improvement over the ICRF-159 and irradiation.

The eight fraction irradiation course combined with ICRF-159 was the most effective strategy for controlling the primary tumor and reducing the incidence of metastases. ICRF-159 has been shown to block progression through the cell cycle in late G_2 or G_2/M.[14,15] This is the stage during which radiosensitivity is maximum. The large fraction delivered in this situation would be expected to reduce the growth fraction. By the time the remaining daily fractions were resumed, reoxygenation would have occurred,[32] possibly augmented by the ICRF-159 induced normalization of the tumor vasculature.[17,19] The overall effect was 94% total tumor control. The effectiveness of the eight fraction radiation course combined with ICRF-159 was not improved by incorporating levamisole into the regimen. We have therefore concluded that a radiation fractionation scheme that includes an initial large fraction with concomitant ICRF-159 therapy is an effective strategy that takes advantage of local x-irradiation to control the primary tumor without increasing the incidence of metastases.

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