Effect of Localized Hyperthermia on TA3Ha Tumor Transplanted Subcutaneously in the Tails of Mice

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ABSTRACT

Localized hyperthermia (43°C) in single or multiple fractions was applied to mouse mammary adenocarcinoma TA3Ha implanted into the s.c. tail tissue of strain A mice. The effects of heat on the growth of local tumors, on the pattern of metastasis, and on the survival periods of the hosts were studied. Hyperthermia was administered by heating the tumor-bearing tails in a water bath. Multiple 30-min hyperthermia treatments at 5- or 7-day intervals controlled local tumor growth better than did a single 30-min treatment or multiple 30-min treatments at 3-day intervals or at intervals longer than 7 days. Heat treatments that produced cytostatic effects on tumors, sparing the normal tissue, had no effect on either the survival of the hosts or the extent of metastasis to the lungs and the lumbar lymph nodes. However, local treatments reduced the frequency of renal lymph node metastasis, indicating that concurrent metastases in different sites may exhibit differential heat sensitivities.

INTRODUCTION

Transplantable mouse tumors in inbred strains of mice have been widely used (19, 27, 29, 34) to study the biological effects of HT either singly or in combination with other treatment modalities (4, 6, 12, 17, 23, 28, 33). These studies have shown that heat may be useful in treating cancers, especially when applied in several fractions (4, 14, 24). However, the cells exposed to sublethal heat doses develop thermotolerance (11, 14, 18, 29, 32). In general, thermotolerance decays slowly over a period of 3 to 7 days. Thus, the cytotoxic effect of heat on tumors may vary depending on the interval between successive heat treatments.

Very little information exists on the effect of localized HT given in multiple fractions of equal heat doses on local tumor growth and distant metastases. Since, in clinical use, HT is likely to be given in multiple fractions, knowledge of this aspect is important. The present communication reports on the effect of single and multiple local HT treatments on the growth of TA3Ha tumors implanted in the tails of mice and on the resulting metastases. Tumors implanted in the tails may be conveniently heat treated by suspending the tumor-bearing tails in a water bath without anesthetizing the hosts or fastening the tumors to supports. The tumors can be accurately measured without unduly manipulating them. The tumors in the tail do not hinder the physical activities of the host. These tumors metastasize spontaneously to pulmonary and extrapulmonary sites (2, 3, 25, 36) in a reproducible manner, so that the effect of HT on the pattern and extent of metastasis may be studied.

MATERIALS AND METHODS

Animals. Female strain A mice obtained from The Jackson Laboratory, Bar Harbor, ME, were used. Mice were 8 to 10 weeks old at the beginning of the experiments and were numbered by ear clipping so that the rate of growth of tumors and survival periods of tumor-bearing mice could be recorded individually.

Tumor Line. Transplantable mouse mammary tumor line TA3Ha in syngeneic strain A mice was used. TA3Ha is a spontaneous mammary adenocarcinoma that originated in a strain A/HeHa mouse in the laboratory of Hauschka et al (13). It was converted to ascites form by Hauschka and maintained from then on by i.p. transplantation. TA3Ha is not immunogenic in strain A mice (9). Upon s.c. transplantation in the flank or the tail of strain A mice, TA3Ha tumor cells form solid tumors.

Preparation of Cells. Ascites Cells, approximately 6 days postimplant, were freshly harvested and were used on the same day for each experiment. Contaminating RBC were separated by Ficoll-Paque (Pharmacia Fine Chemicals, Inc., Piscataway, NJ) gradient centrifugation at 250 × g for 15 min at room temperature. Tumor cells were washed 3 times in Hanks' balanced salt solution (Grand Island Biological Company, Grand Island, NY) and finally suspended in Nutrient Medium F-10 (Grand Island Biological Company) containing 10% fetal bovine serum (Sterile Systems, Inc., Logan, UT).

Tumor Transplantation. Each strain A mouse was given an injection of 10³ TA3Ha tumor cells in 0.05 ml in the s.c. tissue of the tail. Injections were given between the dorsal and lateral veins approximately 3 cm from the distal end of the tail.

Administration of HT. HT treatment was started about 3 days after the tumor appearance. Local HT was administered to tumors in unanesthetized mice by suspending the tumor-bearing tails in a constant-temperature, circulating water bath. The temperature of the water bath was held constant at 43 ± 0.05°C (S. D.). This procedure raised the intratumoral temperature to that of the water bath in about 30 sec. The mean tumor temperature was 43 ± 0.05°C. Control tumors were untreated. The day of first treatment is designated Day 0.

During the treatment, the mice were restrained in 50-ml plastic centrifuge tubes with perforations to allow aeration and to prevent condensation of moisture inside the tubes. Tails were drawn through slits cut in caps of the tubes and suspended through 10-ml syringes. The inside diameter of the syringe was large enough to accommodate the tumor-bearing tail comfortably. Large perforations were made in the syringes to facilitate proper circulation of water and uniform temperature distri-
bution all around the tumor. To insulate the rest of the body from exposure to heat, the plastic tubes with mice inside were placed on a styrofoam sheet with only the tails protruding into the water.

In the study in which a single HT treatment was administered, it was at 43° for 30, 60, or 90 min. When multiple treatments were administered, they were at 43° for 30 min, repeated at various intervals as will be discussed under "Results." When 2 heat treatments were administrated for studying the kinetics of development and decay of thermotolerance, unequal heat doses were used. The first was a priming treatment for 30 min at 43°, and the second was a challenge treatment for 60 min at 43°. The day of challenge is designated Day 0. Control tumors in this study were treated only once at 43° for 60 min. The treatments were scheduled so that all challenge treatments were given on the same day.

Evaluation of the Treatment Effect. The effect of HT on the primary tumors was evaluated by analyzing their rate of growth. Tumors were measured at 3 in CM orthogonal diameters (a, b, c) from which the GMD was calculated (GMD = \(\sqrt{abc}\)), as described by Sharkey and Fogh (31). GMD and standard deviations were calculated for each group. The student t test and the Wilcoxon rank sum test were used for statistical evaluation of the results. Careful autopsy was performed on every dead mouse, and the sites showing macroscopic metastases were noted.

RESULTS

Effects of Heat on Local Tumor Growth. Single HT treatment for 30 or 60 min at 43° resulted in erythema and induration in the tumor area, which persisted for 24 to 48 hr. The inflammatory reaction following a 90-min treatment was much more severe, and partial tail loss was also seen (Table 1). Histological examination of the tails excised 24 hr after a 30-min treatment showed no visible damage to the normal tissue. In contrast, the tumor area showed signs of necrosis. No host mortality was seen as a result of HT treatment.

Chart 1 shows the effect of single heat treatment for 30 and 60 min on the growth of TASHa tumors. Tumors treated for 30 min were generally smaller than the control tumors but not significantly so. Increasing the duration of treatment to 60 min resulted in a significant retardation of tumor growth, compared to the controls (\(p < 0.05\) to \(p < 0.001\) between Days 5 and 15). However, at the time of host death, the treated tumors were not significantly smaller than were the control tumors. A 90-min treatment appeared to retard the tumor growth even further, but at the time of host death these tumors were not significantly smaller than the control tumors. Heat treatment for 30 or 60 min did not prolong the survival of the hosts, but a 90-min treatment increased the host survival (\(p < 0.01\)) compared to the controls (Table 1). Since it was not possible to increase the heat treatment beyond 90 min without toxic effects to the normal tail tissue, we investigated the effect of fractionated heat treatments.

When HT was administered in fractions of 30 min at 43°, it was possible to expose the tails for at least up to 240 min (highest heat dose studied) without any visible effects on the normal tail tissue. Chart 2 shows the effect of multiple heat treatments on the tumors, when administered repeatedly at intervals of 3, 5, or 7 days. Mice in Groups 1 and 2 received 2 and 3 treatments, respectively, at 3-day intervals. Heat treatments in these 2 groups had no significant cytotoxic effect. Mice in Group 3 received 8 treatments at 3-day intervals. These tumors were significantly smaller than were the controls after 5

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effect of single heat treatment on TASHa tumors in the tails of mice</th>
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<tbody>
<tr>
<td>Group</td>
<td>No. of mice</td>
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<tr>
<td>1. Control</td>
<td>10</td>
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<td>2. 30 min</td>
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<td>3. 60 min</td>
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<td>4. 90 min</td>
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\(^a\) From the day of tumor inoculation. 
\(^b\) Mean ± S.E.
treatments \((p < 0.05; p < 0.001\) after 8 treatments).

Mice in Groups 4 and 5 received 3 and 5 treatments, respectively, at 5-day intervals. The tumors thus treated were significantly smaller than the controls \((p < 0.05)\) after only 2 treatments. Similarly, the tumors treated at 7-day intervals (Group 6) also were significantly smaller than were the controls \((p < 0.05)\) after 2 treatments. These tumors were smaller than the tumors treated 3 times at 3-day intervals from Day 15 \((p < 0.01)\) to the day of host death \((p < 0.01)\). Increasing the intervals between heat treatments to 10 or 14 days was less effective than the treatments at either 5- or 7-day intervals (data not shown).

It seemed possible from these above observations that the TA3Ha tumors acquired transient heat-induced thermal resistance (thermotolerance) which decayed over a period of 5 to 7 days. In order to investigate this further, TA3Ha tumors primed with a heat treatment for 30 min at 43° were challenged with a second heat treatment for 60 min at 43°. The effect of heat on the tumors was evaluated based on the relative tumor sizes \((i.e., \text{GMD on Day X/GMD on Day 0})\). Results are shown in Chart 3. The tumors challenged 3 days after the priming treatment seemed to grow unaffected by the challenge treatment, whereas those in the other groups showed retarded growth for 4 to 6 days. The tumors challenged 3 days after the priming treatment increased in size significantly \((p < 0.05)\) from Day 0 to Day 7, while those in the other groups did not. These results indicated that the less pronounced effect of HT when administered at intervals of 3 days was probably due to thermal resistance of a transient nature.

### Effect of Localized HT on Tumor Metastasis

Untreated TA3Ha tumors in the tails of strain A mice spontaneously metastasized predominantly to the lungs, LLN, and RLN. Metastases were less often seen in the mediastinal lymph nodes and the spleen. Metastasis to the liver and ovary was rarely seen.

Autopsy examination of the control and the treated mice immediately after their death revealed extensive lung metastasis accompanied by bleeding into the pleural cavity. These lung tumors were small and innumerable, in contrast to large discrete nodules seen in mice given injections of TA3Ha cells into their tail veins. The sizes and numbers of these tumors precluded enumeration of the lung nodules and thus a quantitative estimate of the extent of lung metastasis. However, since lung metastasis seemed to be the cause of host death, the survival periods of mice were taken as indicators of the severity of lung metastasis. In general, the survival periods of treated mice were no shorter than those in the control mice, indicating that local HT did not enhance lung metastasis. A slight increase in the survival periods of mice treated once for 90 min may be suggestive of a metastasis-inhibiting effect of HT.

The frequency of metastasis to LLN was also 100% in both the treated and control groups of mice. Therefore, the effect of HT on LLN metastasis was evaluated based on the sizes of these tumors at the time of host death. Results are shown in Table 2. No significant differences were seen in the LLN sizes between the control and treated mice.

The effect of HT on RLN metastasis was evaluated based on the frequency of RLN metastasis. No significant increase in RLN metastasis was seen as a result of HT treatments. On the other hand, single HT treatment for 30, 60, and 90 min reduced the frequency of RLN metastasis from 79% in the controls to 60, 50, and 35% respectively (Table 2). Multiple fractions of heat also produced a similar reduction in RLN metastasis. In the group of mice that received 3 HT treatments at 3-day intervals, no reduction in the frequency of RLN metastasis was seen. It
may be noted that, in this group of mice, the local tumor was also unresponsive, possibly due to thermotolerance. No enhancement of metastasis in any of the other organs was evident. Metastasis to the mediastinal lymph nodes appeared to be less frequent in the HT-treated mice compared to the controls. However, since the frequency of metastasis to this site is generally low, no definitive statement could be made on the extent of reduction.

DISCUSSION

These studies were undertaken to investigate the applicability of a tail tumor model to the study of the effects of single and multiple heat treatments on local tumor growth and on the spontaneous metastases from the tail tumor.

TA3Ha tumors grown in the tails of strain A mice seem to offer several advantages for studying the biological effects of localized HT. Some of these are: (a) HT can be easily administered by suspending the tumor-bearing tails in a water bath, eliminating the need to anesthetize the mice or to fasten the tumors to supports; (b) the tails have relatively little fat and fur, which facilitates accurate measurement of the tumors; (c) the tumors can be treated and measured without unduly manipulating them, thus eliminating the possibility of artifactual increase of metastasis; (d) the tail tumors (even large ones) do not hinder the movement of the hosts, in contrast to the leg tumors, so that food and water are available in a true sense ad libitum; and (e) the tail tumors spontaneously metastasize in a reproducible manner, thus permitting studies on the effect of HT on the pattern and extent of metastasis. However, the tail tumor model has seldom been used for such investigations.

Allen (1) studied the effect of HT on Crocker sarcoma transplanted s.c. in the tails of rats. HT was administered by housing the tumor-bearing animals in an oven at 60°–93°. Because of uncontrolled temperatures, accidental death rates, and tail burns, the results are difficult to interpret. Hahn et al. (10) studied the effect of localized HT (44° in a water bath) on the normal tail tissue of BALB/c mice. They found that tail losses occurred due to such treatments, but this could be prevented by administering fractionated heat treatments. Our results also showed that, when heat was administered in several fractions, heat treatment for at least up to 240 min could be delivered without any evidence of toxicity to the normal tail tissue. In contrast, a single heat treatment for 90 min resulted in tail loss.

Fractionated HT affected the growth of the tumors to varying degrees depending both on the total heat dose and on the interval between successive heat treatments. Heat treatments repeated at 3-day intervals seemed to be less effective in controlling the tumor growth compared to those administered at longer intervals. Similar observations were reported by Kim et al. (20) in their clinical studies. They observed that superficial melanomas treated with a combination of HT and radiation responded better when treated at weekly intervals than when treated twice a week. This change in response was suggested to be due to thermotolerance. We found that even with the 3-day treatment schedule, tumor response could be greatly improved by increasing the number of treatments. However, even this improved effect was inferior to that produced by fewer treatments administered at 5-day intervals. This inferior response to heat administered at 3-day intervals may probably be due to persistence of thermotolerance at least for a period of 3 days after heat treatment at 43° for 30 min.

Thermotolerance is generally demonstrated by using 2 unequal heat doses (8, 14, 16, 21). When the TA3Ha tumors exposed to a priming heat treatment were challenged with a larger heat dose after 3, 5, or 7 days, the tumors challenged after 5 or 7 days responded better than did those challenged after 3 days. These results strongly favor the view that the poorer response of tumors exposed to heat at 3-day intervals may be due to thermotolerance.

Dickson and Ellis (5) reported that a single heat treatment of 42° for 60 min to Yoshida sarcoma implanted in rats, resulted in 1 week and increased spread of the tumor. Although this conclusion was based on a small number of rats that survived the heat treatments, it raised a major concern regarding the safety of HT for treating cancers. Studies by other investigators (11, 15, 32) using localized HT under controlled conditions failed to show any metastasis enhancing effect of HT. Since in the TA3Ha tail-tumor system the pattern of metastasis was reproducible and since the tumor manipulation was minimal, we considered this to be an appropriate model for examining the effect of HT on metastasis.

Local HT under the present experimental conditions appeared not to enhance tumor metastasis. On the contrary, it seemed to retard or prevent metastasis even when the heat treatments were not curative. Retardation or inhibition of metastasis to RLN with no such effect on metastasis to LLN was striking. This differential effect of heat on the metastases in different organs may perhaps be due to differential heat sensitivities of tumor cell subpopulations. Existence of tumor cell subpopulations with different heat sensitivities has been recently shown in a human colon carcinoma cell line (22) and in a rat mammary tumor line (35). It has also been demonstrated that different tumor subpopulations may possess differential affinities to specific organs (7, 30). Our more recent studies have indicated that the cells from RLN are indeed more heat sensitive than are those from LLN (26).

It appears from these studies that the tail tumor model may be a very useful one for studying the biological effects of HT on the tumors. In vivo tumors develop thermotolerance following exposure to heat. Thermotolerance seems to persist for at least up to 3 days. Localized HT, at least under the present experimental conditions, did not appear to enhance tumor metastasis. Concurrent metastases at different sites may exhibit differential heat sensitivities.

ACKNOWLEDGMENTS

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