Effects of Hyperthermia on Primary and Metastatic Tumor Growth and Host Immune Response in Rats

Melvin Schechter, Stephen M. Stowe,¹ and Harold Moroson²

ABSTRACT

A hot water bath was used to heat locally a metastasizing carcinoma in Wistar/Furth rats. Applying heat such that intratumor temperature is maintained at a mean value of 42.3°C for two 90-min sessions results in a decreased growth rate of the primary tumor as well as distant metastases. Heating the primary tumor for only one 90-min session or heating the leg contralateral to the tumor-bearing limb has no effect on the growth rate of either the primary tumor or metastases. Heat therapy has no detrimental effect on the spleen cell-mediated tumor immune response of rats as tested by an in vitro lymphocytotoxicity assay 1 day later. However, heating isolated spleen cells to similar temperatures in vitro reduces their capacity for in vitro tumor cell killing.

INTRODUCTION

For over a century it has been repeatedly reported that systemic or local application of heat may be of medical benefit in a variety of diseases, including cancer. The literature contains numerous reports of the arrest or disappearance of malignant tumors after prolonged fevers or of local heating, e.g., the report of Cavaliere et al. (2). Overgaard and Overgaard (13) have provided an extensive literature review of clinical and experimental evidence indicating that tumor cells may be selectively killed by high temperature.

It is generally acknowledged that cellular immune reactions can influence tumor growth but the available evidence on the effects of hyperthermia on host immune response is contradictory. Dickson (4) reported that hyperthermia in rats or rabbits sufficient to cause regression of a primary tumor is accompanied by enhanced metastases. A depression in the immune defense mechanisms of the host at increased body temperature is suggested as a possible cause. In a preliminary report, Schaeffer (14) noted that whole-body hyperthermia increases the spread of artificially induced metastases in mice. The view that heat lowers host immune response is supported by Harris (10), who reported that cytotoxic mouse spleen cells when heated in vitro at 43°C for 45 min lost their capacity to kill specific tumor target cells.

In contrast to the above, there is clinical evidence consistent with hyperthermia stimulating patient antitumor immunity. In some instances of multiple tumor foci, heat treatment of 1 focus resulted in regression of both the treated and untreated tumors (3, 17). Goldberg and Langner (9) investigated hamsters bearing bilateral tumors and reported impaired growth of untreated tumor following heat treatment of the contralateral tumor. Mondovi et al. (12) reported that Ehrlich ascites tumor cells killed by hyperthermia are more immunogenic than cells inactivated by X-irradiation. Stehlin et al. (17) reported that the in vitro cytotoxicity of patient lymphocytes toward their own tumor cells is enhanced after hyperthermia and therefore postulated that heat renders tumor cells more antigenic, stimulating antitumor immunity of the patient. Suit (16) however, in a preliminary report, found heated fibrosarcoma cells less immunogenic in mice than irradiated cells.

In this communication, using a rat metastasizing tumor, both local and distal progression of the disease in response to hyperthermia are correlated with the effects of the therapy on in vitro cell-mediated cytotoxicity, in an attempt to resolve some of the apparent contradictions relative to the effects of hyperthermia on the host immune response.

MATERIALS AND METHODS

Animals and Tumors. The mammary carcinoma, termed Me-H, originally arose spontaneously in a Wistar/Furth rat (Microbiological Associates, Bethesda, Md.) and gave occasional metastases in rats with transplants having large primaries to lymph nodes, pancreas, and lung. The tumor is maintained by i.m. transplantation of tumor homogenate to the hind leg. After several years of passaging in this syngeneic strain, the tumor developed the capacity to rapidly and aggressively metastasize to the retroperitoneal lymph nodes and lung. At its present stage of evolution, the tumor metastasizes early in 100% of the rats but only to the retroperitoneal lymph nodes. Multiple foci are not observed. An injection of 10⁵ cells gives rise to a barely palpable tumor within 7 to 9 days. At this stage there are no gross metastases but if the primary is excised, a metastasis develops within 14 days to the same extent as in intact rats. If left untreated the tumor grows continuously and kills 100% of the rats in 4 to 5 weeks.

The Wistar/Furth rat fibrosarcoma BP-11 was originally induced by benzpyrene. The tumor is maintained by i.m. transplantation of tumor homogenate or cultured cells in syngeneic rats and is 100% transplantable and lethal in 6 to 8 weeks. An injection of 10⁵ cells gives rise to a barely palpable tumor in 10 to 12 days. The tumor is nonmetastasizing and can be controlled by surgical excision before it is more than 2 to 3 cm in diameter. The BP-11 tumor is not immunologically cross-reactive with the Me-H tumor.

Cell Culture. Explants of the Me-H and BP-11 tumor were grown in monolayer culture in Earle’s minimal essential medium containing 10% heat-inactivated fetal calf serum (Associated Biomedic Systems, Buffalo, N. Y.). Assays

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Hyperthermia. Rats with a barely palpable tumor on their right hind leg were anesthetized with sodium pentobarbital (25 mg/kg) and placed on a plexiglass platform resting over a 42.6 to 42.8°C water bath. The temperature of the bath was controlled with a thermoregulator (Arthur H. Thomas Co., Philadelphia, Pa.) sensitive to ±0.1°. The tumor-bearing leg was immersed in the water bath through a hole in the platform, so that the tumor was completely submerged. Temperature monitoring probes (Type 402; Yellow Springs Instrument Co., Yellow Springs, Ohio) were inserted rectally and the temperature was measured at 5-min intervals by means of a multiprobe 6-channel telemeter (Model 44TF; Yellow Springs Instrument Co.). The thermometer is accurate to ±0.12°. The temperature was found to increase with increasing depth of insertion of the rectal temperature-monitoring probe to a maximum of about 9 cm past the anus in 200- to 250-g rats. At this depth the sensor is in the transverse colon and this is the position routinely used. Intratumor temperature was monitored with a 24-gauge hypodermic needle probe (Type 524; Yellow Springs Instrument Co.), positioned so that the temperature sensor was at about the center of the tumor mass. Immediately after hyperthermia, each rat was given an injection i.p. of 1.0 ml of a 37° 0.9% NaCl solution to replace fluid loss. The animal was then placed in a 37° hot-air oven for 30 min to allow body temperature to return to normal. Sham-heated rats were treated in the same manner except that their tumor-bearing leg was immersed in a 37° water bath.

For in vitro heating of rat spleen cells, nonadherent cells were suspended in RPMI-1640 plus 10% heat-inactivated FCS. A test tube containing the cell suspension was placed in a water bath at appropriate temperature for 1 hr.

Cell-mediated Cytotoxicity Assay. Assay is performed by a modification of the method of Bean et al. (1). The plates are incubated at 37° in 5% CO₂ in air for 2 days and then washed 3 times with 37° phosphate-buffered saline (0.01 M Na₂HPO₄·0.14 M NaCl, pH 7.2) containing 0.5% ovalbumin using a modification of the method of Seeger et al. (15). Plates are washed by immersion and gentle movement in the baths. The plates are inverted in the first but not the last 2 rinses to facilitate removal of nonadherent cells (lymphoid cells and nonviable tumor cells). After this, plates are given a final wash in 37° phosphate-buffered saline without ovalbumin, allowed to dry upright, and the bottom of the wells containing labeled tumor cells are punched out and processed for liquid scintillation counting as previously described (1). For prelabeling of tumor target cells, subconfluent monolayer cultures in Falcon 3013 flasks (Falcon Plastics, Oxnard, Calif.) are washed 3 times with Earle’s minimal essential medium (proline free) containing 10% FCS. Five ml of the same medium and 75 µCi of [³¹H]proline (24.5 Ci/m mole; New England Nuclear, Boston, Mass.) are added to the flasks, and the cultures are incubated 20 to 24 hr. After labeling, the cultures are washed twice with RPMI-1640 (containing unlabeled proline), with 10% FCS and detached with 0.2% trypsin. After 2 other washes with RPMI-1640 and 10% FCS, the cells are suspended in this same medium at a concentration of 3 × 10⁶ cells/ml. A 0.1-ml cell suspension (3000 cells) is seeded into the wells of Falcon 3040 microcytotoxicity plates. Prior to addition of effector cells, the labeled target cells are allowed to attach to the well bottom at 37° in 5% CO₂ in air for 1 to 2 hr.

Effect of Hyperthermia on Primary and Metastatic Tumor Growth. In Chart 1, the results of heating of the Me-H tumor-bearing or contralateral limb are compared in terms of the effect on growth of the primary tumor. A single session of hyperthermia to the ipsilateral leg has no significant effect, but 2 sessions on consecutive days result in significant retardation in the rate of tumor growth (p < 0.02 by Day 6; p < 0.003 by Day 8 after initiation of treatment) relative to sham-heated controls. The effect of
Effects of Hyperthermia on Cell-mediated Cytotoxicity.

Spleen cells from rats bearing barely palpable Me-H tumors were heated in vitro for 1 hr at various temperatures (Table 2). Immediately after heating, the cells were tested for their ability to induce detachment of tumor target cells. Heat caused a dramatic reduction in the cytotoxic capacity of spleen cells toward an immunologically specific target. The minimum temperature at which reduced cytotoxicity is observed is $40.5^\circ$, which approximates the temperature adjacent to the rat spleen during the regimen of in vivo heat therapy sufficient to cause a reduction in the rate of tumor growth. Heating spleen cells above $40.5^\circ$ results in further reduction in their cytotoxic capacity but does not affect their viability as judged by the trypan blue exclusion test. In vivo hyperthermia has no significant effect on spleen cell cytotoxicity (Table 3).

**DISCUSSION**

The major conclusions that can be drawn from the data are: (a) local heating of a primary tumor for two 120-min sessions, such that intratumor temperature is maintained at $42.3^\circ$ for 90 min at each session, inhibits the growth of both the primary and a preexisting metastasis distant from the site of therapy; (b) heating inadequate to reduce growth rate of a primary tumor does not result in enhanced growth of metastases; and (c) local heating adequate to inhibit tumor growth has no effect on the host’s cell-mediated immune response in an in vitro assay.

We found that local hyperthermia applied by immersing the tumor-bearing limb in a water bath can cause reduced growth rate of primary, as well as decreased weight of a retroperitoneal metastasis when compared with sham-heated controls. This adds to the body of evidence showing that local hyperthermia can be therapeutic with respect to tumors distant from the site of therapy (3, 9, 15).

Dickson and Ellis (5) reported that local heating of a Yoshida tumor in rats, inadequate to induce regression, causes enhanced spread of metastases. In other communications from their laboratory (4, 6, 7), it was reported that heating of the Yoshida sarcoma in rats of the VX-2 carcino-

**Table 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Wet wt (g) of retroperitoneal metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham hyperthermia (2 times)</td>
<td>2.3 ± 0.4(8)</td>
</tr>
<tr>
<td>Ipsilateral hyperthermia (1 time)</td>
<td>2.2 ± 1.6 (8)</td>
</tr>
<tr>
<td>Ipsilateral hyperthermia (2 times)</td>
<td>1.2 ± 0.5 (6)</td>
</tr>
<tr>
<td>Contralateral hyperthermia (1 time)</td>
<td>3.0 ± 1.9 (8)</td>
</tr>
<tr>
<td>Contralateral hyperthermia (2 times)</td>
<td>2.2 ± 1.2 (7)</td>
</tr>
<tr>
<td>Primary tumor amputated</td>
<td>3.0 ± 1.0 (4)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, number of rats.
* $p < 0.02$ compared with sham hyperthermia.

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**Histology of the Me-H Tumor.** Sections of the primary tumor and nodal metastases reveal that they both have a similar histological pattern, i.e., anaplastic carcinoma with nuclear atypia, multiple mitotic figures and with many nuclei containing several nucleoli. One day after hyperthermia treatment the tumor shows pyknosis, inflammation, and necrosis, indicating cellular injury.
Table 2

Inhibition of cytotoxicity by in vitro heating of spleen cells

Spleen cells from rats bearing a barely palpable Me-H tumor were heated in vitro by 1-hr immersion in a hot water bath at the indicated temperature. Immediately after heating, the cytotoxic capacity of the spleen cells toward Me-H tumor cells and allogeneic BP-11 tumor cells was tested.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Me-H target cells</th>
<th>BP-11 target cells</th>
<th>Me-H target cells</th>
<th>BP-11 target cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm</td>
<td>reductiona</td>
<td>cpm</td>
<td>reduction</td>
</tr>
<tr>
<td>21–23°</td>
<td>1,358 ± 189b</td>
<td>66c</td>
<td>5,351 ± 454</td>
<td>22d</td>
</tr>
<tr>
<td>37.0</td>
<td>1,529 ± 304</td>
<td>62</td>
<td>5,036 ± 479</td>
<td>26c</td>
</tr>
<tr>
<td>39.5</td>
<td></td>
<td></td>
<td>3,950 ± 706</td>
<td>37</td>
</tr>
<tr>
<td>40.5</td>
<td>3,137 ± 428</td>
<td>22a</td>
<td>4,687 ± 310</td>
<td>25a</td>
</tr>
<tr>
<td>41.5</td>
<td>3,878 ± 216</td>
<td>16</td>
<td>6,582 ± 225</td>
<td>4a</td>
</tr>
<tr>
<td>Media control</td>
<td>4,032 ± 779</td>
<td></td>
<td>6,840 ± 1122</td>
<td></td>
</tr>
</tbody>
</table>

a In relation to media control.  
b Mean ³H cpm of 6 samples ± S.D.  
c,d Difference between percentage of reduction of pairs identified with superscript letters are significant at p: c-g, <0.001; g-i, <0.05; f-j, <0.001; d-h, <0.02; and e-k, <0.05.

Table 3

Effects of in vivo hyperthermia on spleen cell cytotoxicity

Rats bearing Me-H tumor were subjected to 2 sessions, on consecutive days, of local hyperthermia to tumor-bearing limb. Either 1 or 2 days after the last hyperthermia session, spleens were removed for assay. Measurement was made of spleen cell cytotoxicity toward specific and nonspecific tumor target cells.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Me-H target cell</th>
<th>BP-11 target cell</th>
<th>Me-H target cell</th>
<th>BP-11 target cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm</td>
<td>% reductiona</td>
<td>cpm</td>
<td>% reduction</td>
</tr>
<tr>
<td>1 Day posthyperthermia</td>
<td>48.3 ± 18.3(4)b</td>
<td>29.0 ± 5.3(4)</td>
<td>61.8 ± 14.2(4) b</td>
<td>9.8 ± 2.5(4)</td>
</tr>
<tr>
<td>1 Day post-sham hyperthermia</td>
<td>70.7 ± 5.8(3)</td>
<td>30.0 ± 7.5(3)</td>
<td>64.7 ± 6.0(3)</td>
<td>11.0 ± 6.2(3)</td>
</tr>
<tr>
<td>2 Days posthyperthermia</td>
<td>71.8 ± 3.8(4)</td>
<td>6.0 ± 4.5(4)</td>
<td>37.0 ± 11.2(4)</td>
<td>12.5 ± 7.1(4)</td>
</tr>
<tr>
<td>2 Days post-sham hyperthermia</td>
<td>74.3 ± 6.7(3)</td>
<td>7.0 ± 7.0(3)</td>
<td>40.3 ± 19.7(3)</td>
<td>9.3 ± 16.0(3)</td>
</tr>
</tbody>
</table>

a Mean percentage of cytotoxicity (relative to media control) ± S.D.  
b Numbers in parentheses, number of spleen donors. Each spleen was assayed separately and 6 replicate assays were done with each spleen.
modes of therapy generated roughly equivalent primary intratumor temperatures. This suggests that the enhanced spread of metastases in response to hyperthermia probably does not result from inadequate heating of the tumor, but rather from overheating of the host.

It is not clear whether the inhibitory effect of hyperthermia on growth of metastases reported in this communication is a manifestation of an immune response triggered by hyperthermia. We found that heating tumor-bearing rats in vivo has no effect on spleen cell-mediated cytotoxicity toward specific tumor cells as tested in an in vitro assay. Heating was such that the temperature in the transverse colon adjacent to the spleen was raised to 40.7° for 1 hr. Spleen ther mia. We found that heating tumor-bearing rats in vivo a manifestation of an immune response triggered by hyper ther mia consistent with viability of the host has no adverse effect on this facet of the tumor immune response.

In our hands hyperthermia has no direct effect on the immune system sufficient to influence tumor growth. This is evidenced by the fact that heat applied to the limb contralateral to the tumor-bearing leg has no effect on growth rate of either the primary or secondary tumor. Hyperthermia may, however, have an indirect stimulatory effect on host immune response by causing increased antigenicity in heated tumor cells (12). Experiments are now in progress to determine whether the inhibition in growth of metastases observed when the primary tumor is heated is also observed in rats immunosuppressed by whole-body X-irradiation.

REFERENCES


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