HYPERTHERMIA: HEALING AND HAZARD IN TUMOR BEARING RATS

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Abstract

We have treated a metatasizing tumor in the rat with hyperthermia, as well as the contralateral normal limb by immersion in a 43 C bath. Whole body (rectal) temperatures were allowed to reach 40.5 - 41.0 C for one hour, and possible harmful effects on the host immune system investigated. Treatment which caused regression of the primary tumor also significantly reduced extent of retroperitoneal metastases. Lymphocytotoxicity against tumor target cells *in vitro* assayed one and two days after hyperthermia revealed no reduction in cell mediated immunity.

Introduction

Medical healing of cancer by hyperthermia results from the ability of microwaves or other physical means to elevate temperature in tumor tissue. The lethal effect of heat on cancer cells is well recognized, with general agreement that tumor cells are preferentially killed in comparison with normal cells[1]. Nevertheless heating normal cells, particularly cells involved in the host immune-response, may be hazardous and excessive whole body heating is usually avoided. Whole body temperature may rise due to blood circulating through the heated tumor. The rise in body temperature depends upon the degree of imbalance between heat introduced and heat lost. If the rise in whole body temperature due to blood circulation from the treated area is excessive hyperthermia may suppress the host immune function. This study was undertaken to investigate the possible harmful effect on metastatic growth following local tumor hyperthermia treatment of an aggressively metastasizing mammary carcinoma, termed ME/h, transplanted into syngeneic Wistar/Furth rats. The contralateral leg in a group of rats was heated to determine the effect of whole body heating on primary tumor growth.

Historical

Schaeffer treated ostogenic sarcoma in mice and found that heating the primary tumor enhanced the number of metastatic colonies in mouse lungs[2]. Dickson and Ellis[3] reported a more rapid dissemination of malignant Yoshida sarcoma cells in rats which survived heating at 42 C for 24 hours or longer, compared with sham heated controls. A possible depression in immune response at increased body temperatures was suggested as being responsible for the more rapid spread of disease[4]. Direct physical evidence of the thermal hazard to hos lymphocytes was provided by Harris[5] in an *in vitro* study of mouse lymphocytes capable of killing mastocytoma target cells. Harris demonstrated that lymphocyte cytotoxic ability was dramatically decreased by heating to 42-43 C for 25 minutes. Under these heating conditions he suggested that thermal destruction of lymphocytes membrane function may have occurred, although under these same heating conditions, the tumor cells were not more susceptible to immune lysis by unheated lymphocytes.

Because hyperthermia enhances the action of X-rays, it has been suggested as an adjunct to radiation therapy[6]. While there is a good basis for the synergistic effect of heat and ionizing radiation[7] on tumor, since ionizing radiation is immunosuppressive, there also may be a hazardous synergism with heat on immunosuppression. There is little hard data on this subject, so we have examined the effect of hyperthermia upon a metastatic primary tumor by measuring: a) growth of primary tumor, b) growth of retroperitoneal metastases, and c) cytotoxic potential of lymphocytes taken one and two days after hyperthermia. Harris' *in vitro* experiment with heat sensitivity of cytotoxic lymphocytes was also repeated with our system.

Materials and Methods

Microcytotoxicity assay.

The microcytotoxicity assay, which measures tumor specific immunity in man, was first described by Takasugi and Klein[8] and subsequently modified by the Hellstroms[9]. The visual counting of cells was replaced by radioisotope counting in the method of Bean, *et al*[10]. In this assay, known numbers of syngeneic and allogeneic tumor target cells previously established in monolayer tissue culture, and labelled with ^{3}H-proline are seeded into microwells and exposed to lymphocytes from immune animals, with lymphocytes from normal animals serving as controls. Detachment of the target cells after incubation for 48 hours indicates cell death.

Target cells in log phase growth phase are labelled for 24 hours with ^{3}H-proline (Sp. act. 35 Ci/mmole, New England Nuclear). Following labelling cultures are washed thoroughly with Hanks balanced salt solution (HBSS), trypsinized, counted, and 10,000 cells introduced into each well of the microcytotoxicity plate (Falcon Plastics 3040), assuming a plating efficiency of 80%, as determined in earlier experiments. Effector spleen lymphocytes from tumor bearing and normal rats are added at an effector target cell ratio of 200:1. After incubation for 48 hours, the microcytotoxicity plate is gently inverted and washed in PBS containing 10% calf serum to remove detached cells, allowed to dry, and the bottoms of the wells punched out with a punch press, and collected in glass scintillation vials. Tissue solubilizer and toluene containing fluors are added, and the vials counted in a Packard Tri-Carb liquid scintillation counter. The percentage cytotoxicity is calculated from the formula:

$$\left(1 - \frac{\text{Av. cpm medium control} - \text{Av. cpm effector cells}}{\text{Av. cpm medium control}}\right) \times 100$$

Hyperthermia.

A temperature controlled water bath is fitted with a plastic top containing 4 circular holes for heating 4 rats simultaneously, and a clamp permitting 4 rat legs to be gently fixed while heating. Rats are anesthetized with 25 mg/kg dosage of Nembutal.

Rats were implanted with tumor and when tumor is palpable (usually day 10-11 post-transplant) divided into 5 groups of 8 rats each. The first group (control) received sham hyperthermia, that is anesthesia, and tumor-bearing limbs immersed in a 37° bath for 1 hour; the second group

(control) had their contralateral limb (non-tumor bearing) treated with hyperthermia (43°C bath, rectal temperature 40.5-41.0C for 1 hour) on one or two days; the third group received hyperthermia to the tumor-bearing limb, rectal temperature 40.5-41.0C for 1 hour, on one or two days.

Tumors and Target Cells.

The cell line ME 254 is derived from a spontaneously arising mammary carcinoma in a W/Fu rat which originally metastasized to lymph nodes, mesentery, and lung, and was adapted to grow in tissue culture in MEM medium (ABS Inc., Buffalo, N.Y.). Following repeated passage (12-14 X) in culture, this cell line is malignant but no longer metastasizes. When the tumor is passaged by a tumor mush (.1 ml) from a tumor bearing rat, it is termed ME/h and is aggressively metastasizing to retroperitoneal cavity, and to lung. A subline of this tumor adapted to tissue culture, RF grows as a benign pleomorphic sarcoma, which eventually regresses spontaneously. Inhibition of tumor growth is determined by caliper measurements of orthogonal diameters of the tumor daily after treatment.

Purification of Lymphoid Cells.

Spleens were removed, cells expressed by mincing, spun down, and the red cells lysed in Tris-NH_4Cl, with removal of adherent polys and macrophages by incubating the leukocytes in plastic flasks at 37C for one hour. The non-adherent lymphocytes were collected and counted.

Temperature Measurement.

Rectal (whole body) temperatures are monitored with a 12 channel YSI Telethermometer and thermistor probes. A needle thermistor was used to monitor tumor temperature which was generally .5C lower than the bath.

Results

Hyperthermia treatment of the tumor bearing limbs caused tumor regression, as shown in Figure 1, while treatment of the contralateral limb caused enhancement of primary tumor growth, compared with sham hyperthermia treatment. Hence the whole body heating schedule was sufficient to cause tumor growth acceleration. After four days the heated tumor resumed its normal rate of growth. There were no cures attributed to the treatment. The two treatment schedule was more effective than the one treatment schedule.

One week after hyperthermia treatment the rats were all killed, and autopsied for metatastic deposits. Visible retroperitoneal masses were excised, and their wet weight determined. Rats receiving local tumor hyperthermia demonstrated a significant reduction in the weight of metastatic deposits, as seen in Chart 1.

In vitro cytotoxicity.

Cytotoxic lymphocytes were tested *in vitro* by hyperthermia at temperatures of 25, 37, 40.5, and 41.5C for one hour then immediately assayed for the effect of hyperthermia upon their cytotoxic activity. In agreement with Harris, their cytotoxic potential was drastically reduced, even at the lower temperatures we employed and using a different assay system. These results are shown in Chart 2.

In vivo cytotoxicity.

When the effect of *in vivo* cytotoxicity was assayed, following hyperthermia of tumor at 43C, and whole body (rectal) temperature of 40.7C x one hour, a different picture emerged. That is, there was no significant decrease in lymphocytotoxicity when assayed one day, or two days after hyperthermia. This data is shown in Chart 3. One explanation is that thermal damage to lymphocytes is capable of being repaired *in vivo*, but not *in vitro*.

Conclusions

Whole body hyperthermia of a primary rat tumor which normally metastasizes is healing not only to the primary, but also against metastases in the retroperitoneal cavity. Whole body hyperthermia from local heating of the non-tumor bearing leg causes some enhancement in growth of the primary tumor. Whole body hyperthermia at a temperature which is healing to the primary does not significantly enhance nor depress immune cell-mediated cytotoxicity. *In vitro* hyperthermia of immune lymphocytes does depress cell-mediated cytotoxicity. The discrepancy between the effect on lymphocytotoxicity of *in vivo* heating and *in vitro* heating suggests repair or repopulation of heat damaged lymphocytes take place *in vivo*.

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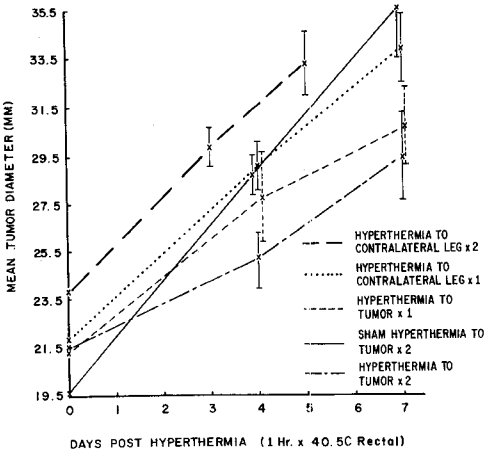

Fig. 1 Rate of growth of primary ME/h tumor in leg of W/Fu rats following 1 or 2 local hyperthermia treatments of tumor bearing leg or contralateral leg.

Hyperthermia treatment	cpm ± s.d. target cells ME/ad	BP-11	% Cytotoxicity target cells ME/ad	BP-11
nil lymphocytes	4032 ± 779	6840 ± 1122	--	--
25.0C x 1 hour	1358 ± 189	5351 ± 454	66	22
37.2C x 1 hour	1529 ± 304	5038 ± 479	62	26
40.5C x 1 hour	3137 ± 428	---	22	--
41.5C x 1 hour	3878 ± 216	6582 ± 225	4	4

Chart 2. Effect of hyperthermia of spleen cells in vitro upon cytotoxicity. Lymphocytes from ME/h tumor-bearing W/Fu rats. Ratio of lymphocytes to target cells is 200:1.

Treatment	Exp. 1 % Cytotoxicity ± s.d. target cells ME/h	BP-11	Exp. 2 % Cytotoxicity ± s.d. target cells ME/h	BP-11
one day post hyperthermia	48.3 ± 18.3	29.0 ± 5.3	61.8 ± 14.2	9.8 ± 2.5
one day post sham	70.7 ± 5.8	30.0 ± 7.5	64.7 ± 6.0	11.0 ± 6.2
two days post hyperthermia	71.8 ± 3.8	6.0 ± 4.5	37.0 ± 11.2	12.5 ± 7.1
two days post sham	74.3 ± 6.7	7.0 ± 7.0	40.3 ± 19.7	9.3 ± 16.0

Chart 3. Effect of hyperthermia (40.7C x 1 hr) of rat tumor in vivo upon cytotoxicity. Lymphocytes taken from tumor-bearing W/Fu rats as shown after heating. Ratio of lymphocytes to target cells is 200:1.

Treatment	No. Rats with Metastases / No. Rats treated Exp. I	Exp. II	Mean Wet Weight of Metastases, gm ± s.d. Exp. I	Exp. II
sham hyperthermia	4/4	8/8	2.4 ± 1.0	2.3 ± 0.4
hyperthermia to contralateral leg x 1 hr.	3/4	7/8	2.1 ± 0.6	3.0 ± 1.9
hyperthermia to contralateral leg x 2 hr.	3/4	7/7	1.5 ± 0.4	2.2 ± 1.2
hyperthermia to tumor x 1 hr	3/3	7/8	2.7 ± 1.8	2.2 ± 1.6
hyperthermia to tumor x 2 hr	1/3	6/6	2.2	1.2 ± 0.5
amputation of tumor	---	4/4	---	3.0 ± 1.0

Chart 1. Effect of in vivo tumor hyperthermia on incidence and extent of metastases in Furth rats with ME/h tumor. Sacrificed 1 week post hyperthermia (40.7C rectal temperature) X 1 hr. or X 2 hr. Controls received either sham or contralateral leg hyperthermia.