Characterization of Mild Whole-Body Hyperthermia Protocols Using Human Breast, Ovarian, and Colon Tumors Grown in Severe Combined Immunodeficient Mice

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ABSTRACT

Objective: We have shown that one treatment of fever-like whole-body hyperthermia (WBH) on mice bearing human breast tumors results in a tumor growth delay. Our goal was to repeat this study in mice bearing human ovarian or colon tumors. We further evaluated this WBH protocol by performing multiple and interrupted WBH treatments.

Methods: Human tumors were grown in severe combined immunodeficient (SCID) mice. For WBH, core body temperatures were maintained at 39.8±0.2°C for 6–8 hours. Multiple treatments were given 6–7 days apart. Interrupted WBH consisted of three 2-hour heatings, 15 minutes apart. Tumor growth time (TGT) was the number of days to grow 1.5 or 2 times in volume.

Results: For WBH-treated ovarian tumors, TGT was 12±1.2d, compared with 5.0±0.1d for untreated mice (P < 0.05). For colon tumors with one WBH treatment TGT was 4.4±1.1d. Two and three treatments had TGTs of 9±2.3d and 8±1.6d. For the untreated tumors, TGT was 2±0.7d (P < 0.01 for one, two, and three treatments). Histological examination indicated that one and two treatments were associated with cellular damage within the tumors. With a slower growing colon tumor, the TGT was 24±3.3d with three WBH treatments, compared with 14±1.8d for controls (P < 0.01). The TGT of breast tumors treated with interrupted WBH was not significantly different than the noninterrupted, with TGT of 7.3±0.8d and 6.2±1.0d, respectively.

Conclusions: These data illustrate that WBH causes a tumor growth delay in mice bearing human ovarian and colon tumors. This response is enhanced with a second treatment of WBH. Interrupted and noninterrupted WBH give comparable anti-tumor results. We will continue to evaluate WBH in various animal models to optimize its potential for clinical administration and maximize the anti-tumor response. Infect. Dis. Obstet. Gynecol. 7:91–97, 1999. © 1999 Wiley-Liss, Inc.

KEY WORDS
hyperthermia; breast tumors; ovarian tumors; immune response; heat shock proteins

For many solid tumors, it is possible to control the initial malignancy with surgical excision. Unfortunately, surgical methods are not as successful if the cancer recurs or spreads to other sites in the body. Although there are already systematic treatments, such as chemotherapy, applied to most patients as follow up to surgery, it is evident that only a small fraction of patients actually benefit...
from this treatment in terms of overall survival. The goal of our laboratory is to develop better therapies to enhance a patient's own immune response against his or her tumor; these therapies may be useful alone or as an adjuvant therapy in combination with chemotherapy or radiation. A large part of our effort is being devoted to the examination of mild, long-duration (fever-like) whole-body hyperthermia (WBH) as a possible means to help stimulate the immune system.1–3

When clinical hyperthermia was first conceived for use in cancer treatment, it was envisioned as a type of thermal radiation therapy whereby a heat treatment sufficient to sterilize or directly kill cancer cells would selectively be delivered to the tumor. It has been noted in the past few years, however, that the tumor temperatures achieved during these local treatments were well below those known to be cytotoxic.1–6 Whole-body hyperthermia protocols have been developed that have employed temperatures as high as 42°C given for durations ranging from 15 min. to 2 hours.7–10 For the studies described herein, we utilized a mild (fever-like) WBH protocol in which the core body temperature is maintained at 39.8°C for 6–8 hours.1,3 This is similar to that obtained during a normal febrile episode, and other work in our lab is devoted to determining what immunologic effector activity is affected by the thermal element of fever.2,11,12

We have previously shown that a single treatment of mild WBH caused a significant (albeit temporary) reduction of tumor volume and a tumor growth delay in severe combined immunodeficient (SCID) mice bearing human breast xenografts and Balb/c mice bearing transplantable, syngeneic tumors.1 The anti-tumor effect seen with the treatment was correlated with the appearance of large numbers of apoptotic tumor cells and an increase in the size of intratumor blood vessels. Substantial levels of several heat shock proteins (Hsps), including Hsp70 and Hsp110, are also induced in the tumor.12 Increased numbers of granulocytes, macrophages, and lymphocytes were also observed in the tumor vasculature and in the tumor stroma immediately following WBH exposure. Our laboratory has also shown that WBH results in several changes in tissue lymphocytes indicative of altered activation levels. These include the alterations in cellular and subcellular proteins, adhesion, and the induction of Hsps.2,11,12 These data provide evidence that there may in fact be an enhancement of the immune system with mild WBH. It is likely that these alterations in structure and function of immune cells that we have recognized are associated with the tumor killing we have observed.

In this paper, we describe experiments to further characterize this fever-like WBH protocol. We have expanded our studies to include human ovarian and colon tumor xenografts. We have investigated the potential to enhance the observed antitumor response with additional treatments of WBH. Lastly, we have altered the protocol to give WBH in two short increments, instead of one long treatment, so that it may be more clinically tolerable.

SUBJECTS AND METHODS
Tumor Implantation and Growth
Human ovarian (#8436), colon (#9427), and breast (#8600) tumor surgical specimens were received from the Roswell Park Cancer Institute Tissue Procurement Facility (Buffalo, NY). These tissues were implanted subcutaneously into 8-week old female c.b.17 scid/scid mice from Taconic Laboratories (Germantown, PA). These tumors were passed several times in SCID mice and dissected into pieces 2 mm in diameter, which were implanted subcutaneously into SCID mice for use in these experiments. After the tumors reached a volume of approximately 60 mm, WBH was initiated.5 The tumors were measured with a vernier caliper every 2 days to determine the shortest diameter (A) and the longest diameter (B). The volume was then calculated by using the formula $V = \frac{A^2B}{2}$. The relative tumor growth ($T_r$) was then calculated for each tumor by dividing the volume at any given day after WBH ($T_d$) by the volume at the start of WBH ($T_o$).

WBH Treatment
Mice were placed in microisolater cages preheated to 39°C that contained food, bedding, and water; it is important that the water was at least 39°C. The cages were then placed in a gravity convection oven (Memmert model BE500, East Troy, WI) with preheated incoming fresh air. The average body temperature of the mice before WBH was 37.5°C. The body temperature was gradually in-
WBH AND TUMOR GROWTH DELAY

REPASKY ET AL.

Fig. 1. Effect of WBH on the growth of human ovarian tumor #8436. SCID mice bearing a human ovarian tumor were treated with WBH for 6 hours on day 0. Tumors were measured with calipers every other day and relative tumor volume was calculated and graphed vs. days post-WBH (see 'Materials and Methods'). In the control group (a; n = 5), TGT was 5.0 ± 0.1d. In the WBH treated group (b; n = 5), TGT was 12.0 ± 1.2d (P < 0.05 when compared with the control; Student t test).

RESULTS

Effect of WBH on SCID Mice Bearing Human Ovarian Tumors

Whole-body hyperthermia was effective in exerting an anti-tumor response on SCID mice bearing human ovarian tumors (Fig. 1). The TGT for this experiment was the number of days for the tumor to reach 1.5 times its original volume. The TGT of the unheated control tumors for this experiment was 5.0 ± 0.1d. The TGT of the WBH-treated tumor was 12±1.2d (P < 0.05). These results are comparable to our previous data using human breast tumors.
Effect of Single and Multiple Treatments of WBH on Mice Bearing a Fast-Growing and a Slow-Growing Variant of a Human Colon Tumor

To investigate the effects of WBH on fast- and slow-growing tumors, we utilized a colon tumor that has both a fast- and slow-growing variant. The tumors in the mice bearing the fast-growing variant of a human colon tumor responded to WBH (Fig. 2a). The TGT for this tumor was the number of days for the tumor to reach 1.5 times its original volume. The control TGT was 2 ± 0.7d. The TGT for the tumors treated one time with WBH was 4.4 ± 1.1d (P < 0.01). Those that were given a second treatment also responded to hyperthermia, and the anti-tumor response was greater (TGT was 9 ± 2.3d, P < 0.01 when compared with the control). For the mice that were given a third treatment, the response was not significantly different when compared with those that received two treatments (TGT was 8 ± 1.6 days, P < 0.01 when compared with control) (Fig. 2b). Histological examination of
the tumors indicated that the first and second treatments of hyperthermia were associated with changes indicative of cellular damage; following a third treatment, tumor structure was comparable to the nonheated control (data not shown). The induction of Hsp70 and Hsp110 was also seen in these tumors following WBH (data not shown). The extent of the anti-tumor responses that have been observed differ from tumor to tumor. For example, when tumor growth was evaluated for a slow-growing variant of the same human colon tumor the TGT was 25 ± 3.3d. When WBH was administered, the TGT was 14.3 ± 1.8d, P < 0.01 (Fig. 3). For the fast-growing variant, the time to reach 1.5 times the original volume was increased by 300% with the treatment. Whole-body hyperthermia on the slow-growing tumor increased the TGT by 108%.

**DISCUSSION**

We have repeatedly observed that fever-like WBH exerts an anti-tumor effect on the growth of human tumors in SCID mice. Human breast, ovarian, and colon tumors respond to this therapy in a similar manner, indicating that the temporary tumor growth delay is most likely not tumor specific. The extent of this response, however, does show some variation between tumors. This difference could be attributed to inherent differences between tumors. This data is highly suggestive that applications of this mild WBH protocol in a clinical setting may be beneficial to patients. Although the result is temporary, it may be enough to give an advantage to current strategies, such as chemotherapy or radiation therapy, which are not always as effective as one would hope. Also, many of these regimens exert toxic side effects at the high doses necessary for success; perhaps WBH could act as an adjuvant to allow these therapies to be given at less toxic doses that would still be effective. Moreover, since our data indicate that WBH is most likely immuno-stimulatory, combining hyperthermia with different types of immunotherapy, such as cytokine
therapy or gene therapy, may prove to be even more successful.

Although combining WBH with other therapies is an ultimate goal, we are continually reexamining the effects of this model alone in order to obtain the greatest possible anti-tumor response. Thus far, our data has shown that 6 hours of WBH will give this maximum response. Interestingly, this treatment can be given in one long administration or in interrupted treatments. The interrupted WBH design could prove to be a more comfortable alternative to patients undergoing WBH. We have also shown that a second 6-hour WBH treatment appears to enhance the anti-tumor response observed with one treatment. However, for at least one model, a third WBH exhibited no apparent advantage over two treatments. Our laboratory is currently investigating possible reasons for this lack of efficacy. We speculate that thermotolerance may have a role; some permanent change in tumor structure may occur with the first and second treatments that result in the loss of response to more heat.

Current studies are also underway to demonstrate interdependence between the anti-tumor response seen with WBH and the changes our studies have shown on both lymphocytes and tumor cells. We have hypothesized that simple mild hyperthermia treatment may have the ability to activate antigen-independent pathways that result in enhanced lymphocyte polarity and adhesion. This in turn may facilitate subsequent antigen-dependent immune events. It is tempting to speculate that these same alterations may also occur during an actual fever following infection or other perturbations of the immune system. Since our data also indicate that even those mild WBH protocols can induce Hsps, we are very interested in determining what their role may be in stimulating the immune response.

A phase-I WBH clinical protocol has recently begun at Roswell Park Cancer Institute (under the direction of W. Kraybill, MD, Department of Surgery). It was designed to evaluate the safety and immunological effects of fever-like WBH alone in patients with advanced cancer. We will also continue to evaluate this fever-like WBH model preclinically in animal models. An understanding of the immune mechanisms that may be enhanced and their possible role in the anti-tumor response seen with WBH could lead to the development of novel therapies in the treatment of human breast, ovarian, and colon cancers.

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