A Proposal for the Addition of Hyperthermia to Treatment Regimens for Acute and Chronic Leukemia

By H. Ian Robins, Warren H. Dennis, Richard A. Steeves, and Paul M. Sondel

The application of hyperthermia to the treatment of neoplastic disease has focused on solid tumors. Human leukemias, both acute and chronic, may represent a unique category of diseases for which hyperthermia should be used in combination with other modalities with curative intent. Three clinical approaches to include hyperthermia are proposed. These are hyperthermia in combination with therapeutic low-dose whole-body irradiation, ablative high-dose total body irradiation, and bone marrow transplantation with the in vitro use of hyperthermia to purge remission bone marrow of abnormal cells. Current preclinical research further supporting these clinical applications of hyperthermia to leukemia therapy is presented.

The potential use of hyperthermia as a treatment modality for cancer is predicated on observation that several types of cancer cells are more sensitive to temperatures >41 °C than their normal cell counterparts.1-3

Beyond this direct killing of neoplastic cells by heat, there is evidence that hyperthermia can be synergistically combined with drugs (anesthetic agents,9,9 chemotherapeutic agents,10-12 interferon13,14) as well as radiation.15-18 Anesthetic agents enhance the killing of cells by hyperthermia.5,9 The hyperthermia potentiation of radiation, chemotherapy, or immunomodulators is probably due to hyperthermia effects other than direct tumoricidal activity.

Concepts developed in the study of killing of cells by heat alone, e.g., optimal temperature and duration of treatment, cannot be directly applied to hyperthermia in combination with other modalities. This point is illustrated by recent work of Mivechi and Hofer19 demonstrating that heat potentiation of radiation lethality and direct heat death are two distinct phenomena (e.g., thermal radiosensitization requires shorter periods of hyperthermia exposure and lower temperatures than direct heat killing). Recognition of such subtle points should have profound implications in the design of preclinical experiments and the planning of clinical protocols. Our prejudice is to use a multi-modality approach to neoplastic diseases. Implicit in the multimodality approach is the awareness that neoplastic diseases are heterogeneous in regard to each cell subpopulation’s response to a given therapy. Hence, such a combined approach diminishes the chances of any subgroup of cells being resistant to all modes of killing.19-25

Neoplastic diseases, refractory to conventional therapy, usually are systemic in nature. For this reason we include whole-body hyperthermia (WBH) in our experimental approach. In this regard, we have recently developed a system for accomplishing WBH in the range of 40 to 41.8 °C using a radiant heat technology.20 This system does not require general anesthesia and has not been associated with toxicity in a recently completed phase I clinical trial.27 Using this same radiant heat technology, we conducted a series of 41 to 42 °C WBH studies in the AKR murine leukemia model.

The results of these preclinical studies, as well as current clinical experience, suggests that hyperthermia may potentially be exploited for the treatment of human leukemias.

In this paper, we present evidence that leukemic cells are sensitive to heat, radiation, and
drugs. Based on the observed sensitivities, we propose the use of hyperthermia as an adjunct to the treatment of leukemia in the following three ways: (1) in vitro treatment of stored remission bone marrow with hyperthermia and anesthetic agents (to purge the marrow of leukemic cells) before autologous bone marrow transplantation; (2) ablative whole-body irradiation (WBI) combined with WBH and systemic chemotherapy as the pre-bone marrow transplant cytoreductive therapy; and (3) WBI in tolerable nonablative therapeutic doses in combination with WBH for the treatment of active chronic lymphocytic leukemia (CLL) and acute leukemias in remission.

Additionally, the known effects of hyperthermia on the immune system and immunomodulators suggest to us the need for preclinical studies of the effect of WBH on the immune system as it relates to the "graft versus leukemia effect" and "host versus graft (rejection) reactions" associated with allogenic bone marrow transplantation.

MATERIALS AND METHODS

AKR Murine Leukemia Model

Several desirable features of the AKR leukemia system led to our selection of this preclinical model. First, these leukemia cells are nonimmunogenic in syngeneic hosts;28 second, experiments can be performed with either transplantable AKR leukemia lines or the spontaneous leukemia that develops in 95% of all AKR mice by five to six months of age; third, rapid, quantitative spleen colony assays of clonogenic cell viability are possible; and fourth, the effect of in vitro treatment on leukemic and normal bone marrow cells can also be assayed using this spleen colony methodology.

Spleen Colony Assay

In in vivo experiments, splenectomy of treated mice is performed nine days posttransplantation of leukemic cells.28-31 The spleens are weighed and their weights, which correlate with tumor burden, are used as a first estimate of treatment effect. Leukemic cell suspensions are prepared from the spleens and injected into assay mice. The viability of the cells in suspension is estimated from the number of surface spleen colonies that develop. To assay for normal hematopoietic colony-forming units, assay mice are irradiated prior to cell injection.

The transplantable form of the leukemia grows rapidly and predictably after intravenous injection. Untreated mice typically die on day 9 postsplenectomy (18 days after transplantation of the leukemia).

RESULTS AND DISCUSSION

In Vitro Purging of Remission Bone Marrows of Leukemia Cells

Using two murine leukemia models, L121032 and AKR,6,33 it has been demonstrated (using spleen colony methodology) that leukemic cells are more sensitive to hyperthermia in vitro than their normal cell counterparts. These data foster the speculation that in vitro hyperthermia might be useful for purging stored remission autologous bone marrow of leukemic cells.6,33-35

We have sought methods for potentiating such a differential hyperthermic sensitivity between leukemic and normal bone marrow cells. In several tumor models, hyperthermic killing of neoplastic cells can be potentiated both in vitro36-39 and in vivo39-42 by anesthetic agents. Indeed, the addition of either lidocaine or sodium thiopental to suspensions containing AKR leukemia cells or normal AKR hematopoietic tissue potentiates the hyperthermic (≥41.8 °C) killing of leukemic cells to a greater degree than its effect on normal tissue.39 This further increases the therapeutic ratio, i.e., the ratio of normal cell to leukemic cell kill. In the AKR model, the addition of 0.75 mm of lidocaine to incubation mixtures at either 41.8 °C or 42.5 °C dramatically increases the ratio of the surviving fraction of bone marrow cells to leukemia cells (therapeutic index). After a 90-minute incubation at 41.8 °C without lidocaine, the ratio is 7.8; it increased to 13.1 in the presence of lidocaine.39 At 42.5 °C the ratios were 13.2 and 186.5, respectively.39 Typical data for sodium thiopental studies are shown in Fig 1. When lidocaine and sodium thiopental are added together to an incubation, the effect observed is additive.39 Similar studies on human tissues using soft agar techniques are currently in progress. If results with human cells are in qualitative agreement with results for the AKR system, application to clinical trials in which autologous bone marrow is treated in vitro with hyperthermia and anesthetic agents as part of transplantation programs could be considered.

WBH in Combination With WBI In Vivo-Murine Studies

Results in many different solid tumor models demonstrating that hyperthermia is a potent radiation sensitizer43 has led to our study of this biologic phenomenon in an animal leukemia model. Table 1 summarizes the results obtained after treating seven- to eight-week-old AKR mice on days 5, 6, 7, and 8 after transplantation of 10⁶ syngeneic leukemia cells. Treatments consisted of WBI (100 cGy per day; Caesatron Model E
Thiopental (mmoles/l)

Fig 1. The therapeutic index at 60 minutes of hyperthermia at 41.8 °C for several concentrations of thiopental. The curve is for the convenience of the reader and was drawn by eye. Therapeutic index is calculated as the ratio of the surviving fraction of normal AKR bone marrow cells at 60 minutes by the surviving fraction of AKR leukemia cells exposed under the same conditions. This data is drawn from the Table 2 of reference 39.

irradiator, Atomic Energy of Canada, Ltd, Ottawa, Ontario) and ~41 °C WBI (one hour per day delivered with a radiant heat box).44,45 Enthermics, Inc, Brookfield, Wis) used singly and together (WBH 30 minutes after WBI). These data support the notion that the effect of combining WBI and WBH is supraadditive. Four mice dem-

onstrating a clinical response after the combined therapy were apparently cured of their transplanted leukemia. Nevertheless, these mice eventually developed spontaneous leukemia, which caused their death. The time at which control mice developed spontaneous leukemia corresponds to the 109 days reported here.

The fact that only 40% of animals treated with WBI and WBH had a survival advantage was an expected result in view of the nonhomogenous response of the AKR model to effective chemotherapy.54 In this regard the AKR model appears to mimic human neoplasia. In comparable studies, spleen colony techniques have demonstrated that the combination of WBI and WBH is supraadditive in reducing tumor burden.45,47

In the experiment presented here the relative spleen weight (i.e., average weight of the treated/average weight of nontreated animals) also suggests that the combination of WBI plus WBH is supraadditive. For radiation alone the ratio is 0.78, for heat alone 0.81, and for the combination (WBI + WBH), 0.29. For normal spleen the relative spleen weight is 0.25. It should be noted that the splenectomy performed on an animal to evaluate colony forming units or spleen weight is in itself therapeutic. A small portion of posttherapy residual tumor, ~5% (by spleen colony assay45), is removed by this surgical procedure.

WBH and WBI in the Treatment of Human Leukemias

We propose that the positive interaction between WBI and WBH as demonstrated in the AKR murine system might be applied to clinical trials. First, WBH plus low-dose WBI may potentially be effective as an adjunct therapy for leukemic patients in remission but at great risk for relapse. Second, this combination may be an important addition in the setting of bone marrow transplantation. Current conditioning regimens are often ineffective at ridding the host of residual leukemic cells.48,49 Further, both cyclophosphamide, often used in conjunction with bone marrow transplantation regimens, as well as anthracyclines, which are used in leukemia therapy, are potentiated by hyperthermia.10,11,43,45 These drugs have been incorporated into clinical trials with WBH for the treatment of solid tumors.51,52 In the case of these solid tumors, there

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Median Survival (d)</th>
<th>±95% Confidence Interval (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>9.0 ± 4.7 (9)</td>
<td></td>
</tr>
<tr>
<td>WBI</td>
<td>8.0 ± 3.1 (11)</td>
<td></td>
</tr>
<tr>
<td>WBH</td>
<td>8.0 ± 7.4 (9)</td>
<td></td>
</tr>
<tr>
<td>WBI + WBH*</td>
<td>34.5 ± 47.0 (10)</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Seven- to eight-week-old mice were given tail vein injections of 10⁶ AKR leukemia cells (carried as an ascites line). Starting on day 5, the mice were given WBH (41.8 °C x 1 h) and WBI (100 cGy) for four successive days. Splenectomies were performed on day 9. From the large confidence interval for the animals treated with the mixed modality (WBI + WBH) and from inspection of the data, one can identify two subpopulations: animals not cured (15.0 ± 1.15 [n = 6]) and animals cured (109 ± 35 [n = 4]).

*WBH given 30 minutes after WBI.
HYPERTHERMIA AND LEUKEMIA TREATMENT

has been just concern regarding the additive myelosuppression if WBH (and WBI) is added to more conventional chemotherapy. If used as prebone marrow transplant conditioning, this added myelosuppression would be of no concern, since the goal of bone marrow transplantation is to ablate the marrow (both hematopoietic and leukemic tissue) and then to rescue the patient with bone marrow transplantation. The limiting factor on adding WBH and WBI will be toxicity to other organs.

Combining WBH with WBI may also be relevant to the treatment of active CLL. This suggestion stems, in part, from previous clinical experience with this disease. Johnson reported excellent results in the treatment of active CLL1,3,56 with WBI. In a subsequent Eastern Cooperative Oncology Group (ECOG) trial, patients with CLL were randomized between chemotherapy and WBI. This study confirmed that the malignant lymphocytes were sensitive to irradiation and could be destroyed with minimal doses of 5 to 10 cGy daily. However, the ECOG trial of WBI was associated with precipitous declines in granulocyte and platelet counts. A retrospective analysis of the ECOG trial57 as well as the National Cancer Institute trial provides data supporting the design of a new study to address the problem of the hematologic toxicity encountered by ECOG. In reviewing the ECOG experience, Rubin et al57 suggested that "... future protocols need to be developed that capitalize on the known effectiveness of radiation to induce CLL response. ..." Thus, we propose, based on our preclinical experience, that WBH may represent the necessary adjunct to WBI to improve efficacy and, perhaps, to diminish toxicity. Such a study has recently been approved for clinical trials at the Wisconsin Clinical Cancer Center.

Implicit in the suggestions to incorporate WBH as an adjunct to the treatment of human leukemias is the existence of a methodology for performing WBH in humans that is safe, simple, and cost effective. Such a system based on control of radiant heat energy loss and gain has been developed at the Wisconsin Clinical Cancer Center and is ideally suited for such studies.26,58,59 This system eliminates the need for general anesthesia, does not display toxicities seen with other WBH systems, and is well suited for multiinstitutional trials.

Hyperthermia, Immunology, and Leukemia

Explanations of the antineoplastic effect of hyperthermia have included the postulation of some immune alteration.60 Such immune effects of hyperthermia can be detected with human cells in vitro.61 However, the data presented here suggest that hyperthermia can cure AKR animals of a transplanted leukemia without inducing lifelong antitumor immunity. Specifically, some animals bearing a transplanted AKR leukemia can be cured of this neoplasm by WBH and WBI. On recovery, these animals proceed to develop the spontaneous AKR leukemia at the same time (age) as animals that have not been previously given the transplanted leukemia and "cured" of it by WBH and WBI. Thus the ability of WBH and WBI to eliminate the disseminated transplanted leukemia does not confer any detectable resistance to the spontaneous leukemia.

In this regard, the transplantable and spontaneous AKR leukemias are nonirritogenic28 as it is difficult to detect any immune response against these leukemia cells in genetically identical AKR animals. In other experimental tumor models, appropriate in vivo or in vitro immunization can yield cells that will confer lifelong immunity to a second challenge of that same tumor.52,63 As the AKR animals cured of transplanted leukemia by WBH and WBI all develop spontaneous leukemia, we conclude that no comparable immune mechanism is at work in these animals.

In contrast, immune mechanisms can be used to eradicate the AKR animal of leukemia, but these require the use of immune cells from another mouse strain. For example, a bone marrow transplant into a nonleukemic AKR mouse from an H-2 identical CBA donor will protect that animal from spontaneous leukemia.64 Similarly, if an AKR mouse is injected with leukemia followed by 900 cGy and bone marrow transplantation from an alloactivated CBA animal, it will not have a relapse of the transplanted AKR leukemia. If the transplant donor is a nonleukemic AKR animal, no protection from relapse could be afforded.65,66 This is the animal model that demonstrates the scientific basis for the "graft versus leukemia" effect seen in human bone marrow transplant patients57 and suggests that immune responses to "minor locus" histocom-
compatibility antigens may be associated with an antileukemic immune response. 86 88 In preliminary studies, we can detect greater immunogenicity of in vitro heated human transformed lymphocytes than that of heated normal lymphocytes. 89 If this result is paralleled with human leukemic cells given hyperthermia, then the addition of WBH to pre-bone marrow transplant conditioning regimens may have a tremendous advantage. First, WBH may increase the destruction of the residual leukemic cells, thereby decreasing the chance for leukemic relapse post-bone marrow transplant. Second, WBH is immunosuppressive itself 86 and may decrease the chance of host versus graft (rejection) reactions. Third, WBH may allow for persistent antigenicity of residual leukemic cells, thereby stimulating an effective graft versus leukemia reaction, to decrease the chance of leukemic relapse. All these postulates can be tested in animal models and could easily be incorporated into ongoing trials of human bone marrow transplantation for leukemia.

Finally, there is the issue of in vitro "purg- ing" of bone marrow of residual leukemia prior to autologous bone marrow transplantation. Approaches using monoclonal antibodies (singly) or as combined "cocktails" are now underway. A valid concern is that a small cohort of residual leukemic cells contaminating the marrow being treated may not be sensitive to the in vitro antibody purging technique. This may occur because a fraction of the leukemic cells might not express the antigen recognized by a particular combination of monoclonal antibodies. The addition of in vitro hyperthermic purging, possibly in combination with a local anesthetic agent such as lidocaine (as presented here), is very attractive. Leukemic cells resistant to the monoclonal antibody purging may be heat sensitive, and vice versa. Furthermore, by treating sequentially in vitro, there should be additive kill of leukemia cells without any deleterious synergistic toxicity to the hematopoietic stem cells.

CONCLUSION

On the basis of the results and discussion above, we conclude that hyperthermia may potentially be used as an adjunct in several therapeutic strategies for leukemia. These include (1) the addition of WBH to low-dose WBI and/or chemotherapy for the treatment of acute and chronic leukemia, (2) the purging of residual leukemic cells in autologous bone marrow transplants, and (3) the addition of WBH to high-dose WBI and supralethal chemotherapy as transplant conditioning designed to eliminate residual leukemia cells from leukemia patients receiving an allogeneic (or purged autologous) bone marrow transplant.

ACKNOWLEDGMENTS

We appreciate Dr Paul P. Carbone’s suggestions and review of this manuscript. We wish to recognize the technical contributions of Patricia A. Martin and Ken Miller.

REFERENCES

HYPERTERMIA AND LEUKEMIA TREATMENT


