Phase I Trial of Human Lymphoblastoid Interferon with Whole Body Hyperthermia in Advanced Cancer

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ABSTRACT

Laboratory studies have shown a potential of the biological effects of interferons (IFN) by elevated temperatures (39.5–40.5°C). Based on such observations a Phase I clinical trial involving 17 cancer patients was conducted to assess the toxicity and biological effects of combining whole body hyperthermia (WBH) (40.5°C for 75 min) and IFN. The study design incorporated a treatment schedule which allowed comparisons of WBH alone, to IFN administered i.m., to combinations of the two modalities. Human lymphoblastoid IFN was given for 6 days in weeks, 2, 4, and 6. At least 4 patients were entered at each of three IFN dose levels (1 x 10⁶ units/m²; 3 x 10⁶ units/m²; 10 x 10⁶ units/m²). WBH was delivered on day 1 of week 1, day 6 of week 4, and days 4 and 6 of week 6. IFN was administered 1 h prior to WBH. The schedule used allowed for the development of tachyphylaxis to IFN-induced fever. Maximum temperatures were not significantly higher 24 h post-IFN/WBH than after a comparable number of days of human lymphoblastoid IFN alone. There was no statistically significant difference in toxicity assessments, hematological and hepatic blood parameters, serum IFN levels, or biological response modulation (i.e., 2',5'-oligoadenylate synthetase activity; β₂-microglobulin levels; natural killer cell cytotoxicity, using K562 target cells and Chang cells) 24 h posttreatment between human lymphoblastoid IFN alone or combined modality therapy. No cumulative toxicity was observed in 6 patients receiving maintenance therapy for up to 1 year. Prior preclinical observations, together with the clinical safety reported in this study, encourage further investigation into the interactions between IFNs and hyperthermia.

INTRODUCTION

IFNs, in vivo and in vitro, inhibit the proliferation of many but not all tumor cells (1). The optimal conditions for this antiproliferative effect have not yet been defined. A series of preclinical investigations has suggested that elevated temperature may potentiate the biological effect of various IFNs. Thermal enhancement of the antiproliferative effects of IFN has been demonstrated with Daudi and rat osteosarcoma cells (2, 3). This enhancement occurred with both partially purified, naturally produced IFN and IFN produced by recombinant DNA technology, purified to homogeneity. In other studies, the combination of IFN and local hyperthermia had a greater antitumor effect in vivo than either agent alone, when tested with transplanted Lewis lung carcinoma in mice (4). A supraadditive antiproliferative effect has also been demonstrated between hyperthermia at 39.5°C and IFN-α and IFN-β in human bladder carcinoma cell lines (5). In these studies cell lines with a range of sensitivities to IFN were examined; in each case the antiproliferative activity was increased at the elevated temperature (5). This effect was also correlated with induction of 2',5'-oligoadenylate synthetase (5), known to be an IFN-inducible enzyme (6). Subsequent murine studies also suggested that a supraadditive effect exists between IFN and WBH at 40.5°C (7).

Based on the above observations, a phase I study combining 40.5°C WBH and human lymphoblastoid interferon (IFN-αLy) was initiated (8). The system utilized to induce WBH was based on a radiant heat device used in conjunction with a defined pharmacological approach to sedation (9, 10). Because of the potential pyrogenic effects of the two modalities, there was particular concern to provide information on patient safety for the planning of Phase II clinical trials. This report summarizes the results of this clinical trial involving 17 cancer patients.

MATERIALS AND METHODS

Patient Selection

Patients were required to have a histologically confirmed malignancy either advanced or metastatic with no probability of cure and not amenable to conventional treatment. Patients had to have an Eastern Cooperative Oncology Group (ECOG) performance status of ≤ 2. Written informed consent was obtained from all patients. Adequate bone marrow function (defined as an absolute granulocyte count of >1,500/mm³ and platelet count >100,000/mm³), adequate liver function (total bilirubin, ≤2.0 mg/100 ml; alkaline phosphatase, lactic dehydrogenase, and SGOT <2 times normal), adequate renal function (creatinine, ≤2.0 mg/100 ml; blood urea nitrogen, ≤30 mg/100 ml; and normal urinalysis), and calcium and electrolytes within normal limits were required for entry.

Patients with a history of an allergy to lidocaine, malignant hyperthermia associated with general anesthesia, documented coronary artery disease, history of angina, congestive heart failure, or serious dysrhythmias were excluded. The protocol excluded patients with severely compromised respiratory status, i.e., full pulmonary function tests less than 65% of predicted. Neurological bases for exclusion were central nervous system involvement by tumor, previous spinal cord or brain irradiation, documented or peripheral neuropathy (paraneoplastic or otherwise), or a history of emotional instability.

Patients could not have received cytotoxic chemotherapy or corticosteroids within 4 weeks prior to initiation of treatment on this study.

Pretreatment Evaluation

Evaluation included a complete history and physical examination; chest X-ray; computer-assisted tomographic scan of the chest, abdomen, and brain; a full chemistry and hematologic survey; full pulmonary function tests; electrocardiogram and radionuclide ventriculography.

Full details of the screening of our center's WBH patients have been described previously (9).

WBH System

The system for delivering WBH, which has been described previously (9), is based on a radiant heat technology (Enthermics Medical Systems, Inc., Menominee Falls, WI).
The WBH device consists of a metal chamber into which a patient is inserted using a stretcher. The chamber is heated by a specialized heating cable wrapped around the metal chamber. The chamber is equipped with doors which seal closed when the chamber and hold a soft collar. The patient’s head remains outside the chamber. The soft collar seals the area around the patient’s neck. The soft collar also permits the passage of an i.v. catheter and monitoring cables into the chamber.

Esophageal, rectal, skin, and ambient air temperatures were monitored using Series 700 thermisters (Yellow Springs Instruments, Yellow Spring, OH) in conjunction with a digital thermometer (Model 5810; Digitem, Dayton, OH).

The Series 700 thermisters were calibrated against a resistive temperature device (accuracy, ±0.02°C; Instrulab, Inc., Dayton, OH) from 34-45°C on 3 days prior to each treatment. These data were analyzed using a linear regression method, and corrections were made in 0.01°C steps from 37.00-43.00°C of the observed readings.

WBH Treatment Procedure

Patients were required to have a hematocrit ≥32 ml/dl to undergo WBH; transfusions were permitted to attain this hematocrit up to 24 h prior to WBH. While on study patients were not allowed corticosteroids, nonsteroidal antiinflammatory agents, or aspirin. Acetaminophen was not administered within the 48 h preceding WBH.

During WBH patients generally received 0.75 to 1.0 liter of i.v. 5% dextrose in 0.25 normal saline per h alternated with 5% dextrose in 0.5 normal saline plus ~7.5 meq of potassium chloride per liter. Body weight, urinary output, and electrolytes were monitored to assure that this fluid regimen maintained homeostasis during and after the procedure.

Patients were sedated during WBH with a combination of i.v. thio- pental (~4 mg/min) and i.v. lidocaine (~4 mg/min); the details and rationale for this have been described previously (9). Patients also received incremental boluses of i.v. diazepam (2–5 mg) and i.v. fentanyl (25–50 µg) (9, 12). Two patients in this study were not well sedated with thiopental or diazepam. As described previously the use of i.v. droperidol (2.5–5 mg) given during the first 30 min of WBH therapy was found to aid in sedation during WBH treatments for such patients (12). The drugs used for sedation in the dose ranges described are not known to affect IFN action or the BRM parameters monitored. All patients received nasal oxygen at 2 to 6 liters/min during WBH.

The aim of sedation was to have a quiet, calm patient who could sleep intermittently yet respond lucidly to verbal stimulation, retain laryngeal reflexes, and continue spontaneous respirations at a rate greater than 10 breaths/min. Verbal contact was maintained with all study patients during sedation. Patients had Foley catheters placed ~30 min prior to WBH; these catheters were removed immediately on completion of the WBH treatment. All patients returned to regular inpatient rooms upon treatment conclusion, i.e., a core temperature ±39°C.

IFN-αLy

IFN-αLy (Burroughs-Wellcome, Research Triangle Park, NC) was provided by the Biological Response Modifiers Program, National Cancer Institute. This IFN has a specific activity of 1 × 10⁶ units/mg protein and contains eight distinct IFN-α species (13).

Study Design

Patients were given IFN-αLy by i.m. injection daily for 6 days on weeks 2, 4, and 6. A minimum of 4 patients were entered at each of 3 IFN dose levels (1 × 10⁶ units/m², 3 × 10⁶ units/m², 10 × 10⁶ units/ m²). WBH (40.5°C for 75 min) was delivered on day 1 of each week, days 4 and 6 of week 4, and days 4 and 6 of week 6 (Table 1). When IFN and WBH were given together, IFN was administered 1 h prior to WBH. In order to undergo WBH, patients needed a hematocrit ≥32 ml/dl, platelet count of ≥80,000/mm³, and a total neutrophil count of ≥500/ mm³.

Patients were removed from study for unacceptable toxicity (as defined below) or for disease progression following the first 6 weeks of treatment. In the absence of progressive disease, patients could continue on maintenance therapy consisting of week 6 treatment (IFN-αLy days 1–6 plus WBH on days 4 and 6) repeated every 4 to 5 weeks. The selection of this time frame for maintenance therapy was arbitrary; it was, in part, based on our perception of probable patient compliance.

ECOG performance status was recorded daily. Vital signs were obtained pretreatment and 2, 4, and 6 h after each IFN injection. Electrocardiographs were performed prior to and 24 h after each WBH treatment. Hematological, hepatic, renal, and metabolic function were monitored before (24 h) and 24 and 48 h after each WBH and on days 1, 3, and 6 of IFN-αLy treatment. Hematological parameters, serum glucose, and electrolytes were also obtained at peak temperature during WBH. Tumor assessments were performed prior to study, at completion of week 6, and monthly thereafter. Toxicity was assessed using ECOG criteria (11), except as noted below.

IFN doses were modified for severe (grade III) and intolerable (grade IV) toxic effects. Grade III symptomatology was defined as vomiting or diarrhea inadequately relieved by medication, fatigue necessitating total bedrest, slow menstrual flow, or a >5-10% weight loss from baseline. Grade IV symptomatology included symptoms requiring administration of i.v. fluids, seizures, confusion, or >10% weight loss. Changes in hematological, hepatic, and renal function that were considered severe included a neutrophil count of <500/mm⁴; platelet count of <50,000/mm³; SGOT of >300–600 units/liter; bilirubin of 2.1–2.7 mg/dl; and creatinine of 2.1–2.9 mg/dl. Intolerable laboratory toxic effects were defined as a neutrophil count of <250/mm³; platelet count of <25,000/mm³; SGOT <600 units/liter; bilirubin >2.8 mg/dl; and creatinine >3.0 mg/dl.

IFN-αLy treatments were withheld for any grade III toxicity until the parameter returned to grade I (minimal) or less toxicity. Therapy was then resumed with the dose of IFN-αLy reduced by one dose level.

Patients who developed grade IV toxicity were removed from study and did not receive further IFN. Dose-limiting toxicity was defined as any grade III or IV toxicity which required a dose reduction or cessation of treatment.

BRM and Serum IFN Evaluation and Sampling

The rationale for studying the various BRM parameters listed below relates to our attempt to investigate whether WBH might enhance the known biological effects of IFN (1, 5). The study design in this regard compares results of treatments with WBH alone to IFN alone and to IFN with one WBH treatment (BRM parameters were not studied during week 6 of this protocol in which 2 WBH treatments were combined with IFN).

Specimens were obtained for biological response modification assays before (30 min) and 24 and 48 h after WBH alone, IFN alone, and the combination of IFN/WBH (Table 1). Samples for βM levels were also collected at 6 h following each modality and following the combination treatment. Serum IFN levels were measured only pretreatment and 6 h after each treatment.
and 24 h posttreatment. No BRM parameters were measured during week 6 of treatment.

**Serum IFN Levels**

IFN-α levels were determined by bioassay (14). Samples from patients were assayed by the encephalomyocarditis hemagglutinin yield-reduction assay in the A549 human carcinoma cell line. Briefly, cells were grown to confluency, washed, and incubated with appropriate serum, or IFN standard, before virus was added. After 30 min unattached virus was washed away and cells were incubated for an additional 18–24 h. Cultures were disrupted by freezing and thawing prior to determining hemagglutinin yields. Serial 2-fold dilutions of virus were added to an equal volume of human type O erythrocytes, incubated, and observed after 60 min. IFN titers were expressed as the reciprocal of the dilution corresponding to the end point for the assay of viral product.

**2,5A Synthetase Assay**

Fifty μl of cytoplasmic extract were incubated with 30 μl of poly(rI) (rC) agarose beads (PL Biochemicals, Piscataway, NJ) equilibrated in buffer A [20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 6.9-85 mM KC1-7.5 mM magnesium acetate-10% glycerol] for 30 min at 37°C. The beads were washed twice with buffer B (i.e., buffer A with the addition of 1 mM dithiothreitol) and then incubated in a shaking apparatus with 25 μl of buffer B to which had been added 4.8 mM ATP and 48 μc/μl [2-3H]ATP (Amersham, Arlington Heights, IL) for 20 h at 37°C. The resultant supernatants were digested with 120 units/ml of bacterial alkaline phosphatase (type IIIIR) (Sigma Chemical Co., St. Louis, MO), at 37°C for 90 min. Aliquots (5 μl) were spotted onto 1-cm² squares of DEAE-cellulose paper (Whatman, Clifton, NJ), dried, and washed in eight changes of distilled water. The phosphatase-resistant, negatively charged, labeled material (oligoadenylylate "cores") was eluted from the filters with 0.3 M KC1 and counted in Aquasol (New England Nuclear, North Billerica, MA). Samples lacking cytoplasmic extract were included as controls, and their values were subtracted from the experimental values. The amount of 2-5A core was calculated from the percentage of conversion of [3H]ATP into [3H]2-5A cores. One unit of enzyme activity was defined as the incorporation of 1 pmol ATP/h/10⁵ cells at 37°C.

**β₂-Microglobulin Assay**

A commercially available radioimmunoassay (Pharmacia, Piscataway, NJ) was used for the quantitative determination of β₂M levels in the serum of patients undergoing therapy. Blood collected by venipuncture was allowed to clot and the serum was separated by centrifugation. Samples were then stored at −20°C until assay (samples are stable for at least 1 year at −20°C).

β₂M levels were measured in a quantitative radioimmunoassay in which β₂M in the sample competes with a fixed amount of ¹²⁵I-labeled β₂M for the binding sites of anti-β₂M antibodies covalently bound to Sephadex (Pharmacia) particles. The competitive capacity was then compared with that of β₂M standards of known concentrations.

Serum samples were diluted either 1:20 or 1:50 with assay buffer and mixed with appropriate concentrations of ¹²⁵I-labeled β₂M and Sephadex beads with antibodies to β₂M. The mixture was then incubated for 45 min at room temperature. Thereafter, the bound and free β₂M was separated by centrifugation and the unbound β₂M in the supernatant was removed. The radioactivity of labeled β₂M, bound to the sedimented Sephadex (Pharmacia) particles, was measured with a gamma counter. β₂M standards were similarly diluted, treated, and counted.

**Natural Killer Cell Cytotoxicity Assay**

Preparation of Effector Cells. Peripheral mononuclear cells were obtained by venipuncture. Blood was heparinized (8 units/ml whole blood), diluted with an equal volume of RPMI 1640 plus 10% fetal bovine serum layered over Ficoll-Hypaque, and centrifuged for 30 min at 470 × g and 24°C. Mononuclear cells recovered from the Ficoll-Hypaque medium interface were washed 3 times with RPMI 1640 plus 10% fetal bovine serum and resuspended in tissue culture medium, i.e., RPMI 1640 medium containing 24 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer, 0.01 mg/ml gentamicin, and 10% fetal bovine serum. All peripheral blood mononuclear cells were incubated overnight prior to testing for NK activity.

Using the technique of overnight incubation, we examined the effects of IFN treatment in vivo upon NK cells and ADCC activity.

Chromium Release Assay for Assessment of NK Cell Activity. NK cell activity was assessed in 1-μl ⁵¹Cr release assays using K562 target cells. Maximum and background ⁵¹Cr release were determined by incubating target cells alone and in detergent, respectively. The percentage of specific ⁵¹Cr release was calculated as

$$\frac{cpm \text{ test release} - cpm \text{ spontaneous release}}{cpm \text{ maximum release} - cpm \text{ spontaneous release}} \times 100$$

in which E is the number of effector cells, T is the number of target cells (2000 well/well), S is the cpm spontaneously released, and M is the maximum cpm released by target cells incubated with detergent. The cytotoxicity constant k describes the relationship between the number of effector cells present and the number of target cells lysed and is proportionally related to the number of LU present in an effector cell preparation. [One LU was defined as the number of lymphocytes required to result in 30% specific ⁵¹Cr release.] Using this equation, the ratio of LU values calculated for any two fitted cytotoxicity curves is the same regardless of the LU definition.

Chromium Release Assay for Assessment of Antibody-dependent Cellular Cytotoxicity. Chang cell monolayers were dissociated with trypsin-EDTA; then single cell suspensions were labeled with Na₂⁵¹CrO₄ washed, and added to round-bottom microtiter wells (2 × 10⁵ cells/well) containing sufficient peripheral blood leukocytes to result in effector:target ratios. ADCC was assessed by adding either medium or rabbit anti-Chang cell antiserum (final dilution, 10⁻²) to appropriate wells, respectively. After 4 h, supernatants were harvested using a Titertek collection system (Flow Laboratories, Rockford, MD) and counted in a gamma counter. Percentage of specific ⁵¹Cr release mediated by K-cells (ADCC) was calculated by correcting for SCC against the same target measured in parallel as follows:

$$\text{cpm test release with antiserum present} - \frac{cpm \text{ parallel SCC release}}{cpm \text{ maximum release} - cpm \text{ parallel SCC release}} \times 100$$

Spontaneous and maximum release represents cpm released from target cells incubated in medium or in 3% Triton-X, respectively. Each measurement represents the mean of quadruplicate determinations.

**Statistical Analysis**

Analysis of hematological and blood chemistry data was performed using the Wilcoxon data system (15). Data were pooled by dose level prior to statistical analysis rendering mean, median, standard error of the mean, and coefficient of variance. Clinical and immunological treatment effects of WBH alone, IFN alone, and the combination were assessed using paired t tests, as were comparisons between treatments. Dose effects of IFN were assessed using analysis of variance. All BRM parameters were analyzed using log-transformed data.

**RESULTS**

Seventeen patients were entered on study (Table 2). Twelve patients (4 of 5 at IFN-αLy 1 × 10⁶ units/m², 4 of 5 at 3 × 10⁶ units/m², and 4 of 7 at 10 × 10⁶ units/m²) completed weeks 1,
2, and 4 of treatment at their scheduled dose level and were evaluated for biological response modification parameters. IFN-related myelosuppression, described below, was the only basis for patients not completing therapy at the IFN dose level dictated by protocol.

WBH Toxicity

Of the total 120 WBH treatments performed there were a total of 6 episodes of headache (3 patients), 10 episodes of vomiting (5 patients), and 8 episodes of moderate fatigue (4 patients). These side effects were all resolved within 12 h post-WBH. No cardiac arrhythmias were observed during this study. We observed 6 instances of self-limiting herpes simplex I in 4 patients receiving the combination of IFN and WBH; these occurred within 4 days following WBH.

Monitoring of hematological and chemistry profiles, which included a complete blood count with a differential WBC, prothrombin time, partial thromboplastin time, liver function tests, electrolytes, serum Ca²⁺ and Mg²⁺, and creatinine phosphokinase before (24 h) and after (24 and 48 h) IFN/WBH administration, revealed no major alterations. Similarly, 40.5°C WBH had no effect on IFN suppression of WBC or platelet counts at peak temperature or 24 and 48 h post-WBH. The results observed were consistent with previously reported laboratory values for comparable WBH treatments at 40.5°C (9). A rise in mean serum glucose was noted at the time of peak temperature for WBH alone (week 1), i.e., 231.6 ± 28.2 (SEM) mg/dl or IFN/WBH (week 4), i.e., 270.1 ± 22.6 mg/dl. The serum glucose returned to normal values at 24 h post-WBH. Typical physiological data as well as a time-temperature profile for a 40.5°C WBH treatment is shown in Fig. 1.

IFN-αLy Toxicity

Subjective side effects produced by IFN-αLy were generally dose related and included chills, fever, headache, myalgia, anorexia, and fatigue. Rigors requiring meperidine were observed in one patient receiving IFN-αLy, 3 × 10⁶ units/m². At all doses, fevers were maximum (≤39.1°C) following the first injection of IFN with tachyphylaxis developing by day 6 of treatment. Patients remained afebrile following the initial WBH treatment and except in one patient receiving IFN 10 × 10⁶ units/m², fevers following the day 6 IFN/WBH combination were no greater than those occurring with day 6 IFN alone (Table 3). (Acetaminophen was administered as needed and was discontinued 48 h prior to hyperthermia.)

One patient developed moderate fatigue and a 2-level ECOG performance status decrease following the first IFN/WBH combination treatment. Mean weight loss over the first 6 weeks of therapy was 2.0 kg. There was no occurrence of significant hypotension, hyperthermia, or CNS toxicity. For patients receiving treatment week 6 (two IFN/WBH combined treatments), toxicity trends were the same as those occurring during week 4 of therapy. Moderate fatigue was noted following both combined treatments but it was no worse following the second treatment.

Doses of 1 × 10⁶ units/m² IFN-αLy produced significant hepatic dysfunction in two patients. One patient with liver metastases from colon carcinoma developed persistent fevers (≥39°C) and SGOT (normal range up to 50 units/liter) elevation to 269 units/liter during week 4 requiring interruption of therapy. Shortly thereafter progressive disease was documented by liver scan. The second patient (fibrosarcoma with no known metastatic involvement of the liver) had an SGOT increase from 98 units/liter to 504 units/liter during week 4 requiring interruption of IFN/WBH treatment. Within 4 days the enzyme had normalized to 151 units/liter. In all other patients hepatic dysfunction was mild and elevations of SGOT were reversible (Table 4).

Interferon produced statistically significant decreases in the WBC at all dose levels. Depression of WBC (P = 0.008) and total neutrophil count (P = 0.02) was maximum following 6 days of treatment with IFN-αLy, 10 × 10⁶ units/m². Dose-limiting hematological toxicity occurred at all three dose levels. IFN-αLy, 1 × 10⁶ units/m², produced grade III granulocytopenia (total neutrophil count, 330) in one patient 24 h after the second combined IFN/WBH treatment at week 6. One patient

### Table 2 Patient characteristics (n = 17)

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<thead>
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<th>Characteristic</th>
<th>No. of patients</th>
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<td>Male/female</td>
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<tr>
<td>B. IFN (3 × 10⁶ units/m²)</td>
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<td>C. IFN (10 × 10⁶ units/m²)</td>
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### Table 3 Mean maximum temperature (°C) weeks 1–4 following WBH and/or IFN-αLy

<table>
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<th>IFN dose (× 10⁶ units/m²)</th>
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<td>38.4</td>
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<td>IFN/WBH</td>
<td>38.5</td>
<td>38.6</td>
<td>38.3</td>
<td>38.6</td>
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</table>

Fig. 1. Typical physiological data collected from a patient (group C, 10 × 10⁶ IFN-αLy units/m²) undergoing systemic hyperthermia. The patient received IFN-αLy 1 h prior to the initiation of hyperthermia at time 0.

### Table 4 Mean maximum temperature (°C) weeks 1–4 following WBH and/or IFN-αLy

<table>
<thead>
<tr>
<th>IFN dose (× 10⁶ units/m²)</th>
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<td>IFN/WBH</td>
<td>38.5</td>
<td>38.6</td>
<td>38.3</td>
<td>38.6</td>
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</table>
Responses (Table 6)

Two patients receiving IFN-αLy, $3 \times 10^6$ units/m$^2$, achieved partial responses. A 37-year-old female with (Stage III) nodular lymphoma who had failed therapy with multiple chemotherapeutic regimens had a $>50\%$ decrease in the sum of bidimensional measurements of her lymph node disease 8 weeks after entry onto the study. This response ended during 7 months of maintenance therapy after which progressive disease was noted. Substantial reduction ($\geq 50\%$) in the size of multiple s.c. nodules occurred in a 38-year-old male whose malignant melanoma had previously responded to IFN-γ. After 5 months of maintenance therapy a new testicular mass developed which on biopsy proved to be metastatic disease.

Prolonged periods of stable disease have been achieved in two patients with leiomyosarcoma. One patient (54-year-old male) with metastatic leiomyosarcoma involving the liver discontinued therapy (IFN-αLy, $3 \times 10^6$ units/m$^2$) after 1 year. Stable disease continues in this patient (40+ months), as well as in a second patient (30-year-old female) with leiomyosarcoma metastatic to liver who continues to receive protocol treatment (19+ months) at IFN-αLy $3 \times 10^6$ units/m$^2$.

A 47-year-old male with a history of malignant melanoma, Clark’s Level II, and excision of an axillary node recurrence was entered onto the study without any clinical evidence of disease. He completed 6 weeks of treatment ($3 \times 10^6$ units/m$^2$) and has remained disease free for 3.5 years.

**Biological Response Modification**

The results obtained from BRM assays are summarized in Table 7 and detailed below.

**Serum Interferon Levels.** Detectable levels of interferon appeared in the serum of only one patient following the initial single treatment with 40.5°C WBH. Serum interferon levels were generally dose dependent following interferon administration. Mean peak antiviral titers 6 h after initial IFN-αLy injections of 1, 3, and $10 \times 10^6$ units/m$^2$ were 81, 417, and 1201 IU, respectively. At doses of 3 and $10 \times 10^6$ units/m$^2$, significant serum levels (>240 IU) were seen 24 h following IFN treatment. Serum IFN levels obtained following combination WBH/IFN treatment were not significantly different from those following week 2 day 6 treatment with IFN alone.

**2-5A Synthetase Activity.** Hyperthermia alone did not significantly affect 2-5A synthetase activity in measurements taken 24 h after WBH compared to pretreatment measurements. Posttreatment levels were on average about 2% lower than pretreatment ($P = 0.92$). Six days of IFN-αLy, with or without WBH on day 6, significantly enhanced 2-5A activity (without WBH: mean increase, 5.0-fold, $P < 0.0001$; with WBH: mean increase, 4.7-fold, $P < 0.0001$). The difference between enhancements with and without WBH was not statistically significant ($P = 0.86$). There was a 1.1-fold increase in 2-5A activity

\[
\begin{array}{|c|c|c|}
\hline
\text{Wk 1 (day 1)} & \text{Wk 2 (day 6)} & \text{Wk 4 (day 6)} \\
\hline
\text{IFN dose} & \text{WBH} & +24 \text{h} & \text{IFN} & +24 \text{h} & \text{IFN/WBH} & +24 \text{h} \\
\hline
1 \times 10^6 \text{units/m}^2 & & & & & & \\
WBC (x 10^3/mm^3) & 7.98 & 8.05 & (0.19) & 5.98 & 6.05 & (0.81) & 5.18 & 5.43 & (0.68) \\
SGOT* (units/liter) & 48 & 54 & (0.42) & 98 & 93 & (0.62) & 67 & 165 & (0.41) \\
3 \times 10^6 \text{units/m}^2 & & & & & & \\
WBC & 6.13 & 5.35 & (0.39) & 3.38 & 3.38 & (1.0) & 2.95 & 2.65 & (0.08) \\
SGOT & 22 & 21 & (0.74) & 42 & 50 & (0.7) & 63 & 49 & (0.33) \\
10 \times 10^6 \text{units/m}^2 & & & & & & \\
WBC & 6.6 & 6.4 & (0.28) & 3.0 & 3.1 & (0.83) & 4.4 & 4.1 & (0.74) \\
SGOT & 35 & 30 & (0.31) & 47 & 46 & (0.64) & 65 & 53 & (0.53) \\
\hline
\end{array}
\]

* Normal range up to 50 units/liter.

\* Values 24 h posttherapy; i.e., either IFN or IFN/WBH.

\* Normal range up to 50 units/liter.

\* Paired $t$ test.
over the 24-h period in which WBH was given on day 6 of IFN (P = 0.58).

Serum β2-Microglobulin Levels. Hyperthermia alone did not significantly affect serum β2-M levels in measurements taken 24 h after WBH compared to pretreatment measurement. Posttreatment levels were on average about 5% lower than pretreatment (P = 0.54). Six days of IFN-αLy, with or without WBH on day 6, significantly increased serum β2-M (without WBH: mean increase, 92%; P < 0.0001; with WBH: mean increase, 110%; P = 0.0001). The difference between the enhancements with and without WBH was not statistically significant (P = 0.39). There was no significant enhancement over the 24-h period in which WBH was given on day 6 of IFN (mean increase, 7%; P = 0.32).

NK Cell and ADCC Activity. Hyperthermia alone did not significantly affect NK cell or ADCC activity in measurements taken 24 h after WBH compared to pretreatment measurement. Posttreatment levels of NK activity showed a mean enhancement of 3% when compared to pretreatment (P = 0.89). Posttreatment levels of ADCC activity showed a mean enhancement of 15% when compared to pretreatment (P = 0.54). Six days of IFN-αLy alone enhanced NK cell activity (mean increase, 5%; P = 0.30) and ADCC (mean increase, 54%; P = 0.08). Six days of IFN-αLy plus WBH on day 6 also showed enhancement of these parameters (NK cell activity: mean increase, 80%; P = 0.09; ADCC: mean increase, 110%, P = 0.03). The difference between enhancements with and without WBH was not statistically significant (NK cell activity, P = 0.52; ADCC, P = 0.91). There was no significant enhancement over the 24-h period in which WBH was given on day 6 of IFN (NK cell: mean log enhancement, 0.208, P = 0.11; ADCC: mean log enhancement, 0.19, P = 0.33).

DISCUSSION

Optimal therapeutic effectiveness of IFNs will likely occur in combination with other modalities of treatment. Substantial preclinical rationale exists for clinical evaluation of IFNs and other biologicals in conjunction with other treatment approaches. IFNs, when used in combination with other treatments, may have substantially different biological effects, i.e., either potentiating or inhibitory. Despite preclinical and clinical investigations, the mechanism of antitumor action of IFNs remains unknown. Thus, systematic clinical evaluations will be required to dissect optimal approaches to the combined use of IFNs with other modalities.

Preclinical investigations (2–5, 7) support clinical evaluation of elevated temperature and IFN. The phase I study reported here represents the first clinical study to address the relationship between fever (i.e., 40.5°C WBH) and IFN-αLy. We believe the results of this study, taken collectively, demonstrate the feasibility of combining IFN and WBH. The strategy of initiating WBH after 3 or 6 days of IFN in order to develop tachyphylaxis to IFN-induced fever was successful, as shown in Table 3. The typical toxicities observed in conjunction with IFN-αLy alone were not altered quantitatively or qualitatively by the addition of WBH. Similarly, the minimal side effects observed with 40.5°C WBH were essentially those observed in other studies (9) and were not affected by the addition of IFN-αLy.

Although clinical responses were observed, it is impossible to conjecture on the possible influence of the addition of WBH to IFN-αLy. Similarly, it would be inappropriate to speculate on the possible effect of therapy on the 3 patients who continue to exhibit stable disease (Table 6). It is of historical interest to note that at the turn of the 19th century, Coley reported a series of patients with malignancies responding to the administration of erysipelas endotoxins (16–18). Such bacterial endotoxins are capable of inducing both interferon (19) and fevers comparable to the temperature selected in this study.

Data for BRM were pooled from all three IFN-αLy dose levels. [Analysis of BRM data as a function of IFN-αLy dose resulted in essentially the same qualitative and quantitative results (data not shown).] IFN-αLy augmented in peripheral mononuclear cells two IFN-induced proteins, 2-5A synthetase and an HLA Class I component (β2-microglobulin). These effects were neither potentiated nor inhibited by WBH. IFN-αLy also stimulated immune effector cell function (Table 7). There was a trend toward a greater enhancement of NK cell and ADCC activity from the combination. Whether such a trend could be statistically validated requires further clinical investigation.

WBH at 40.5°C for 75 min does not induce measurable IFN

<table>
<thead>
<tr>
<th>Patient</th>
<th>sex/age</th>
<th>IFN-αLy dose (×10⁶ units/m²)</th>
<th>Disease</th>
<th>Site</th>
<th>Response</th>
<th>Duration</th>
<th>No. of months of therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>F/36</td>
<td>10</td>
<td>NPDLL*</td>
<td>Nodes</td>
<td>PR</td>
<td>35 wk</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>M/38</td>
<td>10</td>
<td>Melanoma</td>
<td>Skin</td>
<td>PR</td>
<td>20 wk</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>M/50</td>
<td>3</td>
<td>Leiomyosarcoma</td>
<td>Liver</td>
<td>Stable</td>
<td>39 mo+</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>F/28</td>
<td>10</td>
<td>Leiomysarcoma</td>
<td>Liver, spleen</td>
<td>Stable</td>
<td>19 mo+</td>
<td>19+</td>
<td></td>
</tr>
<tr>
<td>M/50</td>
<td>6</td>
<td>Melanoma</td>
<td>NED</td>
<td>Stable</td>
<td>3.5 yr</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

* NPDLL, stage III nodular poorly differentiated lymphocytic lymphoma; PR, partial remission; NED, no evaluable disease; recurrent disease (right axilla) resected; (primary disease 2 years earlier); therapy was given as an adjuvant to surgery.

Table 7 Response to therapy

<table>
<thead>
<tr>
<th>Treatment</th>
<th>WBH alone pre-Rx vs. 24 h post-Rx</th>
<th>IFN alone pre vs. day 6</th>
<th>IFN/WBH pre vs. day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-5A synthetase</td>
<td>2% (P = 0.92)</td>
<td>500% (P &lt; 0.0001)</td>
<td>470% (P &lt; 0.0001)</td>
</tr>
<tr>
<td>β2-Microglobulin</td>
<td>5% (P = 0.30)</td>
<td>92% (P &lt; 0.0001)</td>
<td>110% (P &lt; 0.0001)</td>
</tr>
<tr>
<td>Natural killer</td>
<td>3% (P = 0.89)</td>
<td>65% (P = 0.02)</td>
<td>80% (P = 0.09)</td>
</tr>
<tr>
<td>Antibody-dependent cellular toxicity</td>
<td>54% (P = 0.54)</td>
<td>110% (P = 0.08)</td>
<td>110% (P = 0.03)</td>
</tr>
<tr>
<td>Serum IFN levels</td>
<td>No significant (P)</td>
<td>↑ Proportional to IFN given</td>
<td>Not different from IFN alone</td>
</tr>
</tbody>
</table>
levels. We also assayed the serum of a patient undergoing 41.8°C WBH alone, as a part of another clinical trial, and similarly did not detect the induction of IFN.

A review of the IFN/WBH-related toxicity at the three dose levels show: at dose level 1 (1 x 10^6 units/m^2 IFN), one of five patients had delays in therapy due to rises in SGOT; at dose level 2 (3 x 10^6 units/m^2 IFN), one of five patients had a protocol-mandated dose reduction due to myelosuppression; at dose level 3 (10 x 10^6 units/m^2 IFN), three of the first five patients entered had dose-limiting myelosuppression. Two additional patients were entered at this level to further clarify toxicity. Based on the above data the maximum tolerated dose recommendation for phase II studies in which IFN is combined with WBH is 3 x 10^6 units/m^2.

Based on the toxicity data obtained from this study, a Phase II trial IFN/WBH for advanced renal cell cancer has been initiated as a cooperative study between Wayne State University and the University of Wisconsin Clinical Cancer Center. Additionally, extended preclinical investigations addressing the effects of hyperthermia and IFNs, alone and in combination, on cell proliferation as well as cell survival are also in progress; variables to be studied include temperature, IFN concentration, duration of IFN exposure, and sequencing of IFN and hyperthermia. Such research efforts may ultimately define a role for use of IFN and hyperthermia in treatment of neoplastic disease processes.

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REFERENCES


Phase I Trial of Human Lymphoblastoid Interferon with Whole Body Hyperthermia in Advanced Cancer


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