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A pilot study of melphalan, tumor necrosis factor-α and 41.8 °C whole-body hyperthermia

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Abstract *Purpose*: To evaluate the feasibility of sequencing (based on preclinical modeling) tumor necrosis factor-α (TNF) at two dose levels with melphalan (L-PAM) and 41.8 °C whole-body hyperthermia (WBH) for 60 min. *Patients and methods*: Nine patients with refractory cancer were treated from October 1995 to June 1997. The study encompassed a total of 20 trimodality treatment courses. Three patients were treated at TNF dose level I (50 μg/m^2) and six patients were treated at TNF dose level II (100 μg/m^2). TNF was delivered as a 24-h intravenous infusion, 48 h prior to the combination of L-PAM and WBH; L-PAM was given over 10 min at target temperature at a dose of 17.5 mg/m^2 based on a previous phase I WBH/L-PAM trial. WBH was administered with an Aquatherm radiant heat device. *Results*: Myelosuppression was the major toxicity associated with therapy, but there were no instances of bleeding or neutropenic fevers. Grade 3 thrombocytopenia was seen with 15% of treatments. Regarding absolute neutrophil count, 15% of treatments were associated with grade 3 toxicity, and 45% with grade 4 toxicity, and regarding white blood cell count, 50% of treatments were associated with grade 3 toxicity and 10% with grade 4 toxicity. The myelosuppression observed was equivalent to that seen in our earlier phase I study of WBH and L-PAM (without TNF). Only mild toxicities (grade 1 or 2) were associated with TNF; these were seen with ≤25% of treatments and included nausea, vomiting, diarrhea, fevers, and headache. There were no instances of hypotension. There was no relationship between toxicities observed and the two TNF dose levels. Mild WBH toxicities were seen with less than 15% of treatments; these included nausea, vomiting, and herpes simplex I. Responses included two complete remissions (malignant melanoma, TNF dose level I; breast cancer, TNF dose level II), and two disease stabilizations (both malignant melanoma, TNF dose level I). *Conclusion*: We conclude that the combination of TNF, L-PAM, and WBH is well tolerated at the dose levels studied. The clinical results justify further clinical investigation for this trimodality treatment approach.

Key words Whole body hyperthermia · Melphalan · Tumor necrosis factor · Melanoma

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Introduction

Significant synergism has been reported for the combination of melphalan (L-PAM), hyperthermia and tumor necrosis factor (TNF) in the clinical setting of limb perfusion therapy for sarcoma and malignant melanoma [1]. Unfortunately, the high concentration (1–2 μg/ml) of TNF utilized in such regional therapy exceeds that which can be achieved systemically by bolus (10–20 ng/ml)

[2] or infusional (90–900 pg/ml) [3] administration. It is now generally recognized that the mechanistic basis for the therapeutic effects of high-dose perfusional TNF relates to dramatic vascular events which have been observed both in vivo and clinically [1, 4]. Low levels of TNF, however, can induce molecular changes that have potential therapeutic implications in the context of combined systemic therapy with hyperthermia and chemotherapeutic agents.

For example, Agarwal et al. [5] have demonstrated in L929 cells that TNF induces the activation of ADP-ribosylation resulting in a consumptive decrease of cellular NAD^+ and ATP. The nadir levels of these nucleotides occur 48 h after TNF administration. It is significant that NAD^+ is the rate-limiting substrate for poly(ADP-ribose) polymerase [6]. This enzyme catalyzes the synthesis of ADP-ribose polymers, and thus plays a major role in DNA repair. Interestingly, hyperthermia has also been shown in vitro and in vivo to decrease the NAD^+ and ATP contents of 41.8 °C heat-treated cells [7]; this decrease, is on the basis of blocked biosynthesis [7]. Thus, it has been hypothesized that under defined sequencing, the combination of TNF and hyperthermia might result in an inhibition of DNA repair (by removal of substrate availability) which should enhance the cytotoxicity of a genotoxic agent such as L-PAM. Beyond this, the general depletion of nucleotide pools caused by combining TNF and hyperthermia might yield results that could also be exploited clinically.

This hypothesis has been tested preclinically by Robins et al. [8]. The cytotoxic interactions of TNF, L-PAM and hyperthermia were studied in vitro (L929 cells) at clinically relevant systemic doses. The optimal sequence, producing trimodality synergism, was TNF preceding 41.8 °C hyperthermia by 48 h, and L-PAM given simultaneously with hyperthermia. Noncytotoxic doses of TNF had a superadditive interaction with L-PAM/hyperthermia. Conversely, noncytotoxic doses of L-PAM had superadditive interactions with TNF followed by hyperthermia. Relative to therapeutic index, this optimal trimodality sequence did not result in increased normal tissue toxicity, i.e. myelosuppression, (compared with L-PAM alone) in nontumor-bearing mice [8]. This observation may relate to the ability of TNF to produce cell kinetic changes, i.e. inhibition of the proliferation of human bone marrow progenitor cells [9]. Hyperthermia-induced myeloprotection, as a result of cytokine induction [10–12], represents an additional factor which may serve to explain these in vivo results.

As a first step to extrapolating these laboratory results to the clinic, a phase I trial of L-PAM and 41.8 °C whole-body hyperthermia (WBH) was conducted [10]. The results demonstrated clinical efficacy as well as no significant toxicity other than the expected myelosuppression. Clinical responses occurred in patients with metastatic melanoma. Of note, L-PAM alone had been previously shown to be an inactive agent for malignant melanoma [13]. Based on this trial [10], as well as prior phase I experience with TNF-α [3, 14, 15], a feasibility study was initiated to evaluate the combination of TNF-α as a 24-h infusion at two dose levels (50 and 100 $\mu g/m^2$) given 48 h prior to WBH (41.8 °C for 60 min) and L-PAM (17.5 mg/m^2) given at peak temperature.

At the time of the study's inception it was already recognized that the existing supply of TNF-α, provided by Knoll Pharmaceutical through the National Cancer Institute (NCI), would expire in the fall of 1997. As Knoll Pharmaceutical planned to discontinue the clinical availability of its TNF after 1997, a consensus agreement was reached between the University of Wisconsin Comprehensive Cancer Center, the NCI (USA) and Boehringer Ingelheim (Germany) to do a two-step feasibility study (including up to nine patients) using the remaining NCI supply of (Knoll) TNF. The purpose of the investigation was to gather relevant data to possibly support further clinical investigation in 1998 with a new supply of TNF provided by Boehringer Ingelheim. This report summarizes the experience with nine patients in the initial trial using the Knoll TNF.

Patients and methods

Patient selection

Patients were required to have histologically confirmed advanced or metastatic malignancy with no probability of cure and not amenable to conventional treatment. Patients also needed an Eastern Cooperative Oncology Group (ECOG) performance status [16] of 1 or better, either measurable or evaluable disease, and a life expectancy of at least 12 weeks. Written informed consent was obtained from all patients. Also required were adequate bone marrow function [white blood cell count (WBC) $\geq 3.0 \times 10^3/\mu l$], hepatic function (total bilirubin ≤ 1.5 mg/100 ml), enzymes less than three times normal (normal alkaline phosphatase laboratory range 35 to 130 U/l, normal lactate dehydrogenase laboratory range 90 to 200 U/l, normal gamma glutamyl transferase range 0 to 50 U/l), and renal function (creatinine < 1.5 mg/dl, or creatinine clearance ≥ 60 ml/min, blood urea nitrogen ≤ 30 mg/dl), with serum calcium and electrolytes within normal limits. Patients were accrued to this study between October 1995 and June 1997 (see demographic profile, Table 1).

Patients with a history of allergy to lidocaine, malignant hyperthermia associated with general anesthesia, documented coronary artery disease, angina, congestive heart failure, or serious dysrhythmias were excluded. The protocol excluded patients with severely compromised respiratory status, i.e. any component of full pulmonary function tests being less than 60% of predicted. Neurologic bases for exclusion were CNS involvement by tumor, previous spinal cord or brain irradiation, documented peripheral neuropathy (paraneoplastic or otherwise), or a history of emotional instability.

Pretreatment evaluation

Evaluation included a complete history and physical examination: chest X-ray; computed axial tomographic scan of the brain and, if indicated, of the chest and abdomen; a chemistry and hematologic survey; pulmonary function tests; ECG; and radionuclide cardiac ventriculography. Full details of the screening of our center's WBH patients have been previously described [10, 17].

Table 1 Demographic profile of patients receiving WBH/L-PAM/TNF

No. of patients	9
Male/female ratio	5/4
Age (years)	
Mean	47
Median	48
Range	30–67
ECOG performance	
0	9
Primary diagnoses	
Breast cancer	2
Malignant melanoma	5
Ovarian cancer	1
Prostate cancer	1
Prior therapy	
Chemotherapy only	5
Immunotherapy	3
Radiotherapy only	1
Hormonal therapy	4
Chemotherapy and radiotherapy	1

WBH treatment procedure

The Aquatherm system for delivering WBH (patented, Cancer Research Institute, New york, N.Y.) has been previously described [10, 18]. During all hyperthermia treatments, patients received nasal oxygen at 2 to 6 l/min. Heart rate, respiratory rate, and cardiac rhythm were continuously monitored with a Space Lab Model 3224 A-monitor (Chatsworth, Calif.). Blood pressure (systolic/diastolic) was monitored at least every 10 min with an Accutor blood pressure monitor (Data Scope Corp, Pramus, N.J.).

Esophageal, rectal, skin, and ambient air temperatures were monitored at 10-min intervals using Series 700 thermistors (Yellow Springs Instruments, Yellow Springs, Ohio) in conjunction with a Digital thermometer (Model 5810; Digitec, Dayton, Ohio). The Series 700 thermistors were calibrated against a platinum-resistance temperature device (accuracy ± 0.02 °C; Instrulab, Dayton, Ohio) from 34 to 45 °C three times before each treatment. These data were analyzed using a linear regression method. Corrections were made in 0.01 °C increments from 37.0 to 43.0 °C of the observed readings. Temperature probes were cleaned pre- and post-WBH with povidone-iodine scrub (United States Pharmacopeia 7.5%), followed by a 20-min soaking in glutaraldehyde, followed by rinses in water and then 70% ethanol.

Patients received 0.75 to 1.0 l of intravenous (IV) 5% dextrose in 0.25 normal saline per hour alternated with 5% dextrose in 0.5 normal saline plus approximately 7.5 meq of potassium chloride per liter. Body weight, urinary output ($\geq$75 ml/h), and electrolytes were monitored to assure fluid and electrolyte homeostasis during and after the procedure. A typical WBH treatment session lasted 4 h, including $\sim$ 1.3 h to reach target temperature, 1 h at 41.8 °C, and a $\sim$ 1 h cooling phase. Posttreatment, patients received normal saline 500 to 1000 ml as needed to maintain systolic blood pressures greater than 90 mm Hg. Patients were sedated during WBH with a combination of IV thiopental ($\sim$ 4 mg/min) and IV lidocaine ($\sim$ 4 mg/min); the details and rationale (including seizure and arrhythmia prophylaxis) for this have been previously described [17]. Patients also received incremental boluses of IV midazolam (2 to 5 mg) and IV fentanyl (25 to 50 μg). Droperidol (2.5 to 5 mg) was administered during the first 30 min of WBH therapy for both its sedative and antiemetic effects. The aim of sedation was to have a patient who could respond to verbal stimulation and continue spontaneous respirations at a rate greater than ten breaths per minute. Patients were returned to regular inpatient rooms after treatment and discharged after 20 to 24 h of observation. After WBH, all patients received 10 to 35 mg of metoclopramide IV as prophylaxis against the gastric stasis effect of thiopental. Patients also received granisetron 1 mg IV and decadron 5 mg IV post-WBH as prophylaxis against chemotherapy emetogenesis.

Treatment plan

Table 2 summarizes the treatment schema. Each cycle of therapy consisted of the administration of TNF on day 1 as a 24-h infusion (50 μg/m^2, three patients, and 100 μg/m^2, six patients). Patients received controlled release ketoprofen 200 mg (provided by Wyeth Laboratories, Philadelphia, Pa.) 24 h and 30 min prior to TNF. Patients were given normal saline (100 to 350 ml/h) to maintain a systolic blood pressure of at least 95 mm Hg. WBH (41.8 °C $\pm$ 0.2 °C for 60 min) was administered on day 4 with L-PAM (17.5 mg/m^2) infused over approximately 10 min, 20 min after achieving target temperature as determined by esophageal and/or axillary temperature probes. The dose of L-PAM was determined in our earlier study [10]. In that study it was shown that WBH did not change the pharmacokinetics of L-PAM. Cycles were to be given once every 4 weeks. The recombinant TNF-α (NSC# 635257) was provided by Knoll Pharmaceutical (Germany), through the National Cancer Institute (NCI), Cancer Therapy Evaluation Program. (The TNF was reassayed by the NCI during the final third of the study and was found to show no change in activity.) The L-PAM was provided by Burroughs Wellcome, N.C.

Patients were required to have a hematocrit of $\geq$ 32%, platelet count $\geq$ 100 000, and WBC $\geq$ 3000/ml to undergo WBH/L-PAM. Transfusions of packed RBCs were permitted up to 24 h prior to WBH/L-PAM. During periods of neutropenia patients received ofloxacin for bacterial prophylaxis. Treatment delays up to 4 weeks were permitted for recovery from myelosuppression.

The protocol as designed had detailed procedures for delay of therapy due to abnormal liver function tests, nausea/vomiting, urologic toxicity, mucosal toxicity, as well as other unspecified toxicities relating to TNF, WBH or L-PAM. These are available on request but are not summarized, as the only toxicity encountered in the study requiring treatment modification was a grade 4 neutropenia dictating a dose reduction of L-PAM of 2.5 mg/m^2.

Posttreatment evaluation and duration of therapy

Patients were monitored at least weekly posttherapy for toxicity. The NCI Common Toxicity Criteria were used to grade toxicities. Responses, i.e. complete response (CR) and partial response (PR), were evaluated using standard ECOG criteria [16]. All patients with absolute neutrophil counts (ANC) below 500/μl received oral ofloxacin (500 mg $\times$ 2/day) until recovery of neutropenia (i.e. ANC 750/μl).

Table 2 Schema

Day 1	Days 2–3	Day 4 (48 h post-TNF)	Weeks 3–4 Reevaluation
TNFα 24-h infusion	Observation and support	WBH + L-PAM WBH 41.8 $\pm$ 0.02 °C $\times$ 60 min L-PAM 17.5 mg/m^2 IV (given at peak temperature)	Responding patients receive additional therapy monthly Stable disease (maximum three cycles) Progressive disease – off study

Dose level I, 50 μg/m^2 (three patients); dose level II, 100 μg/m^2 (six patients)

Patients were removed from protocol with the demonstration of progression of disease after the completion of any cycle of therapy. Patients with stable disease could have up to three cycles of therapy.

Results

There were a total of 20 treatments. Three episodes of indwelling central line infection during non-neutropenic periods requiring antibiotics were encountered. Myelosuppression was the major toxicity associated with therapy, but there were no instances of bacterial infection (except as previously noted), bleeding or neutropenic fevers. Regarding thrombocytopenia, there were nine episodes of grade 1, three episodes of grade 2, and three episodes of grade 3 toxicity. Regarding absolute neutrophil count, there were five episodes of grade 2, three episodes of grade 3, and nine episodes of grade 4 toxicity. Regarding WBC count there were six episodes of grade 2, ten episodes of grade 3, and two episodes of grade 4 toxicity. There was no relationship between myelosuppression and TNF dose level. The mean WBC/platelet count nadirs observed during the first cycle of therapy was $3.0 \pm 2.5/130 \pm 56 \times 10^3/\mu l$. In our prior phase I WBH/L-PAM study at the same dose level of L-PAM, i.e. 17.5 mg/m^2, the mean WBC/platelet nadirs were $2.9 \pm 0.8/132 \pm 21 \times 10^3/\mu l$. Interestingly, one patient with malignant melanoma who had been previously treated in an earlier phase I WBH/L-PAM study and relapsed after achieving a partial remission, as previously reported [10], encountered less myelosuppression with equivalent doses of L-PAM given in combination with TNF as part of this study.

Toxicities related to WBH included three episodes of herpes simplex I (in two patients with a history of cold sores), all occurring within 3 days of WBH, which promptly resolved with the use of acyclovir. There were three episodes of grade 1 nausea, and one episode of grade 2 vomiting. There was also one episode of a grade 1 transient elevation in liver function tests lasting 48 h.

Toxicities related to TNF included five episodes of grade 1, and two episodes of grade 2 nausea: and two episodes of grade 1, and one episode of grade 2 vomiting. There were five episodes of grade 1 and one episode of grade 2 headache, two episodes of grade 2 fever, one episode of grade 1 diarrhea, and one episode of grade 2 rigors. There was no apparent difference in the incidence of toxicity as a function of TNF dose level. Patients demonstrated no significant weight loss or anorexia as a result of therapy. The aggressive use of hydration, and timed released ketoprofen prevented any episodes of hypotension.

Neoplastic disease status post-therapy

Although the primary goal of this study was to evaluate the feasibility of combining TNF, WBH and L-PAM, significant tumor responses were observed:

A 54-year-old male patient (with no prior chemotherapy) who had biopsy-proven malignant melanoma involving multiple lymph nodes only, achieved a partial remission (lasting 465 days) in an earlier phase I WBH/L-PAM study [10], in spite of repeated courses of therapy attempting to consolidate the disease response. The patient relapsed with progressive disease and was entered on this WBH/L-PAM/TNF study at level I. The patient entered a complete remission (documented by CT scans) with a response duration of 828 days. Two other patients at level I (a 54-year-old male with prior chemotherapy and immunotherapy and a 30-year-old female with prior immunotherapy), each with melanoma involving the liver, demonstrated improvement less than a partial remission, justifying three treatment courses per patient. These patients achieved disease stabilizations for 128 and 233 days, respectively.

At TNF level II, two patients with malignant melanoma (with prior immunotherapy and/or chemotherapy) demonstrated progressive disease after the first cycle of therapy. Two female patients (with prior chemotherapy) one with metastatic breast cancer and the other with ovarian cancer demonstrated a short period of disease stabilization of 30 days. A second female patient (aged 39 years) with breast cancer (with prior therapy including cytoxan, 5FU, methotrexate and adriamycin) having metastatic pulmonary disease entered a complete remission lasting 240 days, at the time of disease progression the patient was noted to have brain metastases but no other recurrence.

Finally, a 67-year-old male patient with hormone-refractory prostate cancer involving the bone, may be of interest. This patient demonstrated progressive disease with an increasing prostate-specific antigen (PSA) following the first course of therapy. Based on a prior clinical experience (involving a hormone-refractory prostate cancer patient who had responded to hormonal therapy after progressive disease with WBH see reference 17, patient no.12), the patient was placed on bicalutamide and leuprolide acetate. With the reintroduction of hormonal therapy the patient had a complete normalization of his PSA (from a peak value of 153 mg/ml) and resolution of his skeletal pain which had been of considerable duration (in excess of 10 months).

Discussion

Since its introduction almost a decade ago, the combination of TNF, L-PAM in the setting of limb perfusion hyperthermia continues as a therapeutic modality and focus of clinical investigation [1, 19, 20]. This clinical trial represents the first attempt at the systemic application of these treatment modalities. The current protocol sequencing of these agents discussed above and elsewhere [19] was predicated on preclinical modeling [8], as well as on our earlier phase I experience with WBH and L-PAM [10].

There was no suggestion that TNF added to the toxicity of L-PAM/WBH as shown in our analysis of blood count data. Although anecdotal, our experience in a single patient with metastatic melanoma provides putative support for the hypothesis that TNF increases the therapeutic index of L-PAM/WBH; this patient achieved a more sustained and complete remission (with less myelosuppression) with three cycles of TNF/WBH/L-PAM compared with an earlier remission [10] with three cycles of WBH/L-PAM.

The achievement of a complete remission of visceral disease in a pretreated metastatic breast cancer patient represents another positive anecdotal experience. The fact that the only site of relapse in this patient was the central nervous system (CNS), i.e. metastatic disease to the brain, suggests a CNS/chemotherapy sanctuary zone. This same phenomenon of CNS relapse after chemotherapy and WBH was seen in our previous WBH/L-PAM study [10] in a patient with malignant melanoma. Thus, one can deduce that WBH does not have the ability to affect the blood brain barrier, at least so far as L-PAM is concerned. Parenthetically, a survey of the world's literature [21] as well as our more recent collective experience in a cooperative group, i.e. the Systemic Hyperthermia Oncological Working Group [22], suggest that CNS relapse is an extremely rare occurrence.

The apparent reversal of the hormone-refractory state of a prostate cancer patient posttreatment has been previously observed (but not reported) by our group in relation to WBH alone. Clearly this observation may be serendipitous, and requires further laboratory and/or clinical investigation to be considered meaningful.

Taken collectively, we believe the toxicity and early response data derived from this investigation justify further exploration of this trimodality approach . The study was initially conceived (and now completed) as a two-step pilot investigation using an NCI supply of Knoll TNF (which is no longer available). Based on the previously presented findings, we are currently planning a second generation trial encompassing further dose escalation of a new supply of TNF (Boehringer Ingelheim). It is anticipated that in the context of a subsequent study, biological questions relating to TNF effects on nucleotide pools and DNA repair via poly(ADP-ribose)polymerase, which have direct bearing on L-PAM cytotoxicity, as well as other events leading to cellular decompensation, can and will be addressed.

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