Phase I Trial of Intravenous L-Phenylalanine Mustard plus the Sensitizer Misonidazole

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ABSTRACT

Misonidazole (MISO), a hypoxic cell radiosensitizer, has been shown in vivo to enhance tumor cell killing by melphalan (LPAM) with little or no enhancement of normal tissue injury. A Phase I trial was conducted using MISO p.o. 2 hr before i.v. LPAM. The highest doses used were the single maximum tolerated doses of MISO, 4 g/sq m, and LPAM, 0.6 mg/kg. Thirty-five patients were entered; 30 were evaluable for assessment of hematological toxicity, which was predicted to be the dose-limiting toxicity. The median age was 60 years (range, 28 to 72 years). Mild to moderate nausea and vomiting occurred in 80% of patients. Five developed serious hematological toxicity defined as nadir white blood cell count < 1000/cu mm, platelets < 20,000/cu mm or 4-week posttreatment white blood cell count < 2000/cu mm, platelets < 50,000/cu mm. Four of the toxicities occurred at the LPAM dose of 0.6 mg/kg but were independent of MISO dose. One patient died of infection. Two patients whose tumor demonstrated an objective response to therapy and 10 others with disease stabilization received additional courses. Four patients developed mild MISO neuropathy. Pharmacokinetic studies demonstrated that MISO did not appear to affect the pharmacokinetics of LPAM in plasma. Both LPAM and MISO can be given safely at their individual maximum tolerated dose. This combination will proceed to Phase II trials.

INTRODUCTION

MISO is a 2-nitroimidazole introduced into clinical trials as a sensitizer of hypoxic cells to radiotherapy (10, 18, 19, 26). Rose et al. (20) initially demonstrated that MISO can enhance tumor cell killing by chemotherapy agents in mice. This observation, corroborated by others, has been reviewed recently (17, 21). The sensitization occurs primarily with the bifunctional alkylating agent (7,16,17,20). The mechanism of this interaction as a sensitizer if given simultaneously with or shortly before the chemotherapeutic agent (ER, 1.0 to 1.6) in the mice. Since the normal tissue enhancement was less than the enhancement of tumor cell killing, a kinetic interaction of the drugs had been postulated to be part of the mechanism in mice (21).

A Phase I trial using the combination of p.o. MISO plus LPAM was initiated in August 1981. LPAM was chosen because in the laboratory it appeared to be enhanced as well as any other agent. Additionally, although it is an active drug, it is not used in many first-line chemotherapy regimens and therefore will be a new drug for many patients, even those previously treated with chemotherapy. The design of this trial is somewhat different than the standard Phase I trials in that the maximum tolerated single dose of MISO p.o. (26) and LPAM i.v. (2) were known. This study was designed to see if MISO enhanced the hematological toxicity of LPAM or whether the full dose of both drugs could be administered. We also studied the pharmacokinetics of LPAM to see if MISO altered the plasma pharmacokinetics of LPAM as compared to published data using LPAM i.v. alone (2).

MATERIALS AND METHODS

Patient Selection and Evaluation. All patients had histologically confirmed cancer refractory to conventional treatment or for which no effective therapy was known. The patients had not received radiotherapy or chemotherapy during the 4 weeks prior to treatment (at least 6 weeks elapsed since treatment with a nitrosourea or mitomycin C). All patients had a life expectancy of at least 6 weeks, a Karnofsky performance score of >50%, a WBC of <3500/cu mm, a platelet count of >125,000/cu mm; bilirubin, <2.0 mg/dl; serum glutamicoxaloacetic transaminase <3 times normal; creatinine, <1.5 mg/dl. Informed consent was obtained prior to treatment. Initial patient evaluation included complete history, physical examination, CBC with differential, platelet count, chemistry and electrolyte panels, urinalysis, chest X-ray, and pertinent X-rays to help evaluate tumor response. Patients were to have a weekly CBC after treatment and a complete reevaluation at 4 weeks. Retreatment with drug could be given if the CBC and chemistries were still within the above limits and if the treatment was deemed to be clinically indicated by the patient's physician.

Drug Schedule and Administration. MISO and LPAM were obtained from the Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. MISO was supplied in 500- and 100-mg capsules. The appropriate dose was administered p.o. 2 hr prior to LPAM infusion. LPAM, 100 mg/vial, was reconstituted by the addition of 1 ml of acetic acid alcohol diluent followed by 9 ml of dipotassium phosphate-propylene glycol diluent. Both diluents were provided by the manufacturer (Burroughs Wellcome, N. C.). The appropriate dose was added to 100 to 200 ml of 5% dextrose in water solution U.S.P. and administered by i.v. infusion into a peripheral vein over 15 min. The initial dose of MISO was 1.5 mg/sq m, and that of LPAM was 0.45 mg/kg. The scheme is included in Table 3. At least 3 fully evaluable patients were entered on a given step. Dose escalation was done on a succeeding group of patients if the preceding step was sufficiently nontoxic. The hematological toxicity scoring system used is shown in Table 1. Each patient was scored for hematological toxicity at their count nadir (2 weeks) and at recovery (4 weeks posttreatment white blood cell count < 2000/cu mm, platelets < 50,000/cu mm. Four of the toxicities occurred at the LPAM dose of 0.6 mg/kg but were independent of MISO dose. One patient died of infection. Two patients whose tumor demonstrated an objective response to therapy and 10 others with disease stabilization received additional courses. Four patients developed mild MISO neuropathy. Pharmacokinetic studies demonstrated that MISO did not appear to affect the pharmacokinetics of LPAM in plasma. Both LPAM and MISO can be given safely at their individual maximum tolerated dose. This combination will proceed to Phase II trials.

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weeks). Additional patients were added to a step if: (a) any patient developed Grade 3 toxicity at Week 4; or (c) 3 patients developed Grade 2 toxicity at week 4. The study was to be terminated if, on any step: (a) >50% of patients develop Grade 5 toxicity at any time; (b) >50% of patients develop Grade 4 toxicity at Week 4; (c) >75% of patients develop Grade 3 toxicity at Week 4. To be evaluable for toxicity and response, a patient must have been observed for at least 4 weeks following treatment.

Pharmacokinetic Studies: LPAM. Pharmacokinetic studies were done on 8 patients using a modification of the methods of Chang et al. (6) and Furner et al. (13). Blood samples were obtained at 0 time (start of infusion), at 15 min (end of infusion), and at 20, 30, 40, 50, 60, 90, 120, 180, and 240 min. Blood samples were obtained in 10-ml heparinized tubes. The plasma was separated immediately and frozen at -70° if not processed on the same day. The samples were maintained at 4° if not processed on the same day. The samples were maintained at 4° C. 

Table 1
Toxicity score

Table 2
Patient population (n = 35)

Median age, 60 years (range, 28 to 72).

Table 3
Phase I: LPAM plus MISO

RESULTS
Thirty-five patients were entered onto this study; 5 were evaluable due to early deaths or insufficient follow-up data. One patient, inadvertently given 100 mg of LPAM, died of rapidly progressive disease 4 days after drug administration. The characteristics of the patients are in Table 2.

The dose of drugs, numbers of patients, and hematological toxicity are illustrated in Table 3. Severe toxicity, as judged by a hematological score of 4 or 5 occurred in Steps 2, 3, and 5. Overall, 5 patients had this level of toxicity. In Steps 2, 3, and 5, 4 of the 5 patients with the nadir toxicity are the same patients who had the minor toxicity at 4 weeks. Overall, 7 patients had major or minor toxicity. The one drug-related death (Step 5) occurred in a patient with prior chest wall radiotherapy and 15 months of prior chemotherapy. It can be seen that for all patients given LPAM, 0.6 mg/kg, the toxicity did not appear to increase with an increasing dose of MISO. With small numbers of patients, it is possible that an effect of MISO dose on LPAM toxicity could be missed. Assuming an overall toxicity incidence of 16% (5 of 30), if the strongest trend of increasing toxicity with MISO dose was present (i.e., 0% in the lower-dose MISO group and 30% in the highest dose MISO group), the maximal type 2 error would be 25%.

The average length of prior chemotherapy was 7 months. Four of the 5 patients with Grade 4 or 5 toxicity had over 1 year of chemotherapy; 2 of them also had received radiotherapy.
MISO produces a peripheral neuropathy in approximately 30 to 50% of patients given a total dose of 10.5 to 12 g/sq m (10, 18, 26). A minor neuropathy will be detectable but will not be bothersome to most patients. A moderate neuropathy can be debilitating; therefore, if any patients had any symptoms suggesting neuropathy, the MISO was discontinued. A minor toxicity which was subjective only was encountered in 4 patients at doses of 2.0 to 8.0 g/sq m. One patient given 2 g/sq m developed minimal parasthesias; one given 3 g/sq m complained of numbness of the fingers but received an additional cycle of drugs without problems; a patient given 4 g/sq m complained of unilateral foot numbness; and the patient at 8 g/sq m developed what was felt to be a typical MISO-induced parasthesia of the feet.

Nausea and vomiting occurred in 80% of patients. It was with equal frequency on all steps and was generally of less than 12 hr duration. Two patients developed a drug rash and had the treatment discontinued. No other organ toxicity was encountered.

Pharmacokinetics. The results of the pharmacokinetic analysis are shown in Table 4. Since the percentage of unbound melphalan is relatively small (10 to 20%) and the half-life is short, few patients had measurable values beyond 50 min. There were sufficient data to analyze free melphalan in 6 of the 8 patients.

The steady state volume of distribution was larger for unbound drug. In this study, LPAM was administered i.v. because (a) the drug level could be best controlled. The starting dose was 75% of the maximum i.v. dose used by Alberts et al. (2). The purpose of the study was to see if MISO modified the hematological toxicity of LPAM and to see if MISO altered the pharmacokinetics of LPAM, as compared to previously published results (2). MISO has now been used as a radiosensitizer in many patients (18). It is well absorbed p.o. and has a maximum tolerated single dose of 4 g/sq m (26). MISO was started at a relatively low single dose, 1.5 g/sq m.

The initial animal data (7, 16, 17, 20) suggested that the MISO should be given simultaneously with, or slightly before, the alkylating agent. A 2-hr interval was chosen, because this is the time required for MISO to approach its peak plasma level after p.o. administration (26). Subsequently, it has been shown in mice that maintaining a sustained level of sensitizer which simulates the drug half-life after p.o. dose in humans (13 hr) produces a superior therapeutic index compared to a single pulse of drug (4). In mice, the sustained level produces no enhancement of normal tissue injury while an ER of 1.8 to 2.2 was obtained for tumors. In animal studies, a large dose range of alkylating agents was used. In support of the use of this combination in humans are animal data showing that the ER was either constant over the dose range of alkylating agent used or a higher ER was seen (4). In mice, the sustained level produces no enhancement of normal tissue injury while an ER of approximately 2 for tumors, with no enhancement of normal tissue damage.

The results from this trial demonstrate that LPAM i.v. can be given at full dose (0.6 mg/kg) and MISO can be used at its single-dose maximum tolerated dose (4 g/sq m) without causing excess hematological toxicity. Few patients developed severe hematological toxicity (Grade 4 or 5) at any time during their treatment. Drug treatment was repeated in 12 patients, 2 of whom had a measurable tumor response. Nausea and vomiting were the most frequent toxicities and were generally mild and limited to a few hr. It occurred at all dose levels. Four patients developed mild peripheral sensory neuropathy. It would be expected that more patients would develop it had they received more drug. The incidence of peripheral neuropathy is approximately 30 to 50% of a total MISO dose of 10.5 to 12.0 g/sq m (18, 26). It is

<table>
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<th>Dose</th>
<th>MISO (g/sq m)</th>
<th>LPAM (mg/kg)</th>
<th>No. observed</th>
<th>Peak level (µg/ml)</th>
<th>VDss (liters)</th>
<th>t½ (min)</th>
<th>Clearance (liters/min)</th>
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<td>0.37</td>
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</table>

All patients 2.3 ± 0.6b 22 ± 7 65 ± 30 0.34 ± 0.08 0.33 ± 0.06 101 ± 27 24 ± 8 ± 1.0 2.8

Table 4

Pharmacokinetic parameters

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<th>Dose</th>
<th>LPAM (mg/kg)</th>
<th>No. observed</th>
<th>Peak level (µg/ml)</th>
<th>VDss (liters)</th>
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Table 5

Therapeutic response

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DISCUSSION

Melphalan has been useful clinically for multiple myeloma, lymphoma, ovarian cancer, and breast cancer (12). It has been administered in higher doses to patients with melanoma using isolated limb perfusion (15). These studies suggest that a higher dose may be more effective therapeutically than standard doses of drug. In this study, LPAM was administered i.v. because (a) absorption can be erratic after p.o. drug (1) and (b) time of peak drug level could be best controlled. The starting dose was 75% that of the maximum i.v. dose used by Alberts et al. (2). The

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conceivable that administering MISO on an every-4-week schedule may decrease its toxicity, but it is unlikely that a substantial reduction will be obtained. Therefore, if the 2-nitroimidazole drugs are effective as chemosensitizers, a less toxic drug would be needed. Desmethylmisonidazole, the second of those compounds to enter clinical trial, was somewhat less neurotoxic (8) but not sufficiently so to proceed beyond the Phase I study. A newer drug, SR-2508, which may be one-sixth as neurotoxic as MISO has entered Phase I clinical trials as a radiosensitizer. It appears that it is equally effective in vivo as a sensitizer as MISO when used with alkylating agents (4, 16). It is likely that it will be tried as a chemosensitizer in clinical trials in the future.

The mechanism of chemosensitization has not been fully elucidated (3, 17, 21, 24). Since one possible mechanism was a pharmacokinetic interaction of MISO with LPAM, we examined the pharmacokinetics of some of these patients. The t1/2 values for total LPAM in our study (65 ± 30 min) are similar to that previously obtained by Alberts et al. (108 ± 21 min) for patients treated with i.v. LPAM (2), although our assay methods differed slightly. Thus, if a pharmacokinetic interaction exists, it is probably at most a minor effect. This will be further investigated as part of a randomized Phase II trial using LPAM, with or without MISO. Animal data indicated that single-dose MISO alters the pharmacokinetics of LPAM in part by reducing the body temperature of the mice (3, 17, 25). However, little alteration in pharmacokinetics was seen using multiple doses of MISO in a manner that mimics the plasma profiles in humans. More likely, mechanisms of chemosensitization include an increase in LPAM-induced DNA strand breaks by MISO or partial depletion of intracellular glutathione in hypoxic cells by MISO (24).

This trial has demonstrated that LPAM and MISO can be administered at their maximum tolerated doses with little serious hematological toxicity. Phase II trials are to be initiated with this combination through the Northern California Oncology Group. One will be a randomized Phase II trial for patients with advanced non-oat cell lung cancer, and the other will be single-arm Phase II trials for patients with malignant melanoma. They will use MISO at 4 g/sq m 4 hr prior to LPAM, an interval that appears optimal in vivo. Care will be taken after exceeding a total MISO dose of 8 g/sq m due to the possible development of neuropathy. The individual dose of MISO per treatment will be reduced to 2 g/sq m beyond that point. Since a higher plasma level of sensitizer provides better sensitization, it will be advantageous to have a drug less toxic than MISO. SR-2508, now being evaluated in a Phase I trial as a radiosensitizer, may be such a drug (5). Additional chemotherapeutic agents that may be sensitized by MISO include cyclophosphamide and 1-(2-chloroethyl-3-cyclohexyl-1-nitrosourea) (17), and they are worthy of clinical trials.

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