Enhancement of the Clinical Activity of Melphalan by the Hypoxic Cell Sensitizer Misonidazole

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ABSTRACT

One hundred patients with non-small cell lung cancer were entered by members of the Northern California Oncology Group into a randomized Phase II trial of i.v. melphalan versus i.v. melphalan with concomitant oral misonidazole. The patients had not received prior chemotherapy. Eighty-five patients were evaluable for assessment of response and 89 were evaluable for toxicity analysis. The melphalan/misonidazole group had a superior response rate (two complete and four partial responses among 42 patients or 14%) compared to the melphalan group in which there were no responses among 43 patients (p = 0.024, two-sided Fisher exact test). Since hematological toxicity was equivalent in the two groups, there was an improvement in therapeutic index. Data from 12 patients undergoing pharmacological studies demonstrated that the plasma concentration of melphalan was 25% higher in the misonidazole group, a difference that is not statistically significant. Although the mechanism of interaction has not been fully established, this randomized trial demonstrates that a chemosensitizer can enhance the clinical antitumor activity of an alkylating agent and suggests that chemosensitizers in combination with alkylating agents should be investigated in further clinical trials.

INTRODUCTION

Misonidazole was the first in a series of 2-nitroimidazole compounds to be used as a hypoxic cell radiosensitizer. The radiosensitization is due to the electron affinity of the molecule which enables it to stabilize radiation-induced free radicals (1). For hypoxic cell radiosensitization to occur the sensitizer need be present only at the time of irradiation (2). Misonidazole produces a peripheral neuropathy that severely limits the total dose of drug that can be given. Less toxic analogues of misonidazole are now being investigated in clinical radiation therapy trials (3). The efficacy of this approach remains to be established.

Rose et al. (4) observed that misonidazole enhances the efficacy of alkylating agents in vivo. Although the mechanism of this enhancement has not been fully elucidated, hypoxia is required in order for misonidazole to undergo reductive metabolism (5–9). One or more of the reductive metabolites of misonidazole which are formed in the hypoxia cells is felt to be the active species in chemosensitization (10, 11). Misonidazole has not been demonstrated to have antitumor activity by itself. In the laboratory, chemosensitization has been observed for alkylating agents and nitrosoureas using both in vitro and in vivo endpoints (12, 13). The degree of tumor and normal tissue sensitization observed depends on the dose and schedule of the sensitizer. In mice, a large single dose of misonidazole lowers body temperature and alters the pharmacokinetics of the alkylating agents (14–16). However, when misonidazole was administered to mice in a multiple-dose schedule which reproduced the plasma pharmacokinetic profile seen in humans, there was an enhancement in tumor cell killing without concomitant alteration in pharmacokinetics of the chemotherapeutic agent and without an increase in normal tissue toxicity (14–17).

The enhancement of antitumor activity without a concomitant increase in normal tissue toxicity provides a therapeutic gain. Since alkylating agents are of use in many chemotherapeutic regimens, we investigated the ability of misonidazole to enhance the efficacy of an alkylating agent. Melphalan was selected as the alkylating agent for study, as the drug does not require metabolic activation, and the plasma pharmacokinetics of the drug can, therefore, be readily monitored (18). Although single-agent therapy with alkylating agents has not been shown to be highly effective for patients with lung carcinoma (19, 20) no chemotherapeutic regimen has been demonstrated to produce a clearly superior survival advantage (21–23). Furthermore, Mulcahy and Siemann (24) demonstrated that a chemotherapeutic agent need not have a great deal of activity by itself to be successfully modified by misonidazole. We had previously conducted a Phase I trial of melphalan plus misonidazole (25) to establish the dose schedule of drugs to be used. Of the two responses in the Phase I trial, one occurred among the five patients with lung cancer. In this randomized Phase II trial, patients with non-small lung cancer, who had not received prior chemotherapy, were treated with melphalan alone or with melphalan plus misonidazole.

MATERIALS AND METHODS

Patient Selection and Evaluation. Between June 1983 and November 1985, 100 patients were randomized in this Phase II study of the Northern California Oncology Group. Since a relatively low rate of response was anticipated in the investigational arm (melphalan plus misonidazole), a randomized Phase II design was utilized with melphalan as the control arm. The use of the randomized Phase II design helps to interpret the importance of a low rate of response in the investigational arm and may be useful in situations in which adequate rates of response are not assured even with known "active" therapies (26). Criteria for study entry included pathologically documented non-small cell lung cancer, measurable disease, Karnofsky performance status of at least 70%, and normal white blood cell and platelet counts. Following randomization, 51 patients were assigned to treatment with melphalan, 49 were assigned to treatment with melphalan plus misonidazole. Pretreatment evaluation included: history and physical examination including complete neurological exam, chest X-ray, documentation of the measurable disease site, and hematology and chemistry assessments.

Drug Schedule and Administration; Pharmacokinetic Studies. All drugs were supplied by the Investigational Drug Branch of the National Cancer Institute. Melphalan was dissolved in acid alcohol buffer, diluted to a volume of 100–200 ml with D2O or normal saline, and administered at a dose of 0.6 mg/kg, i.v., immediately after reconstitution over a period of 15 min. Misonidazole at 4 g/m2 was given orally 4 h prior to the melphalan. If three or more doses of misonidazole were given, the third and subsequent doses were reduced to 2 g/m2 due to the risk
of developing neuropathy (27). If neuropathy developed, misonidazole was discontinued but melphalan could be continued in the absence of disease progression at the investigator’s discretion. Treatment was administered every 3 weeks.

Pharmacokinetic data are available for 12 patients, six per group. A blood sample for analysis of misonidazole was obtained 4 h following misonidazole administration which was the time of the initiation of the melphalan infusion; a second sample was obtained about 1 h later. Samples were obtained for the measurement of total and nonprotein bound melphalan for 90 min following the start of the melphalan infusion. Beyond this time the melphalan concentration was below the limits of the assay. Melphalan and misonidazole were analyzed, and pharmacokinetic analysis was performed as previously described (25). Free and total melphalan levels were compared both absolutely and by area-under-the-curve methodologies (28) and analyzed using the two-sample \( t \) test.

For one patient in the chemomodifier group the misonidazole serum concentration was not measured.

Tumor Response, and Toxicity Analysis. Sites of disease were considered measurable if bidimensional measurements could be obtained by physical examination and/or radiographs. Standard criteria of response were utilized: Complete response required disappearance of all evidence of active disease. Partial response required a reduction by at least 50% of the product of the two longest perpendicular diameters of all measurable lesions and either no change in, or partial recalciﬁcation of all osteolytic lesions. Minor response required reduction by between 25 and 50% of the product of the two longest perpendicular diameters of all measurable lesions. Progression of disease required a 25% or greater increase in the product of the two longest perpendicular diameters of any measurable lesion, or the occurrence of a new lesion, or the progression of any osseous lesion. All responses were reviewed without knowledge of the treatment group and scored by best response achieved at any time (by R. W. C.). Toxicity was scored by the treating physician utilizing the standard cooperative group criteria of the National California Oncology Group. Nadir blood counts were routinely performed at any time (by R. W. C.). Toxicity was scored by the treating physician utilizing the standard cooperative group criteria of the National California Oncology Group. Nadir blood counts were routinely performed 10–14 days following treatment.

Statistical analysis for comparison between treatment groups for rates of response was performed using the Fisher exact test (30). Patient characteristics and toxicity were analyzed by the Wilcoxon rank sum test adjusted for ties (31). Survival was calculated for the subgroups using the actuarial method of Kaplan and Meier (31) and differences in survival assessed by the log rank test (32). All tests of statistical significance are two-sided tests.

RESULTS

Patient Characteristics and Response. The median age of patients entered on the study was 59 years (range, 37–78 yr). Sixty-five% of patients were males; 77% had a Karnofsky performance status of 80–100%. Twenty % of patients had received prior radiation therapy to the measurable site of disease and all had progressive disease at that site at the time of study entry. One patient who responded to therapy with melphalan plus misonidazole had received prior radiation to the measurable site, a neck mass. The proportion of eligible patients (n = 85) with each histology was: squamous cell carcinoma, 28%; adenocarcinoma, 53%; large cell carcinoma, 19%. There was no statistically signiﬁcant difference between the two treatment groups in any of these variables. Of the 51 patients entered in the melphalan arm two were not eligible on pathology review, six were not evaluable for response (two, no chest X-rays taken; one, refused treatment after the initial dose with response not evaluated; one, responded to radiotherapy at the time of study entry; two, did not receive treatment) leaving 43 evaluable for response analysis. Of the 49 patients in the melphalan/misonidazole arm, three were not eligible (one, probable breast cancer primary; one, central nervous system metastasis; and one, inadequate baseline studies) and four were not evaluable for response (one, only received misonidazole; one, received no treatment; two, received treatment but were not subsequently evaluated for response) leaving 42 evaluable for response analysis.

The response data are shown in Table I which includes the best response obtained by the individual patient. The difference in response rate between the two groups (14% for melphalan/misonidazole versus 0% for melphalan) was statistically significant at the \( p = 0.024 \) level (two-sided, Fisher exact test). The characteristics of the responding patients are in Table 2. In the melphalan/misonidazole arm there were four responses (CR-1) among 21 patients with adenocarcinoma and two responses (CR-1) among 11 patients with squamous cell carcinoma. The response sites are listed the Table 2. The median survival in the melphalan arm was 5.5 months and in the melphalan/misonidazole arm 4.5 months (\( p = 0.34 \)). In the chemosensitizer group the median actuarial survival of the responders is in excess of 25 months while the median survival for the nonresponders is approximately 4 months (\( p = 0.0004 \)).

Pharmacokinetics. The data from measurement of plasma pharmacokinetics are in Table 3. Comparing the total melphalan concentration of each group, the concentration of total melphalan was 25% higher than in the melphalan alone group. Similarly, the pharmacokinetics are in Table 3. Comparing the total melphalan concentration of each group, the concentration of total melphalan in the chemosensitizer group was approximately 25% higher than in the melphalan alone group. Similarly, the pharmacokinetics are in Table 3. Comparing the total melphalan concentration of each group, the concentration of total melphalan in the chemosensitizer group was approximately 25% higher than in the melphalan alone group. Similarly, the

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<th>Table 2 Patient characteristics (evaluable for toxicity)</th>
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* Difference, \( p = 0.024 \) (two-sided, Fisher exact test).
concentration of unbound melphalan is higher in the chemosensitizer group. However the assay loses accuracy below 0.10 μg/ml so that comparison of unbound melphalan values at time points after 35 min might not be valid. No statistically significant differences were observed between the free or total melphalan levels comparing the two treatment groups either by analysis of plasma levels or area under the concentration versus time curve. The 90-min total melphalan level difference was the only one suggestive for statistical significance (0.05 < p < 0.10 without adjusting for multiple comparisons).

The plasma concentration of misonidazole was in the range of 40 to 160 μg/ml, with four of five patients having a value of at least 100 μg/ml.

Toxicity (Table 4). Forty-seven patients in the melphalan arm and 42 in the melphalan/misonidazole arm were evaluable for toxicity analysis. The nonhematological toxicity was generally similar between the groups except for neurotoxicity and nausea and vomiting both of which were more frequent in the chemosensitizer group (neuropathy, p = 0.12; nausea, p < 0.001). There was no difference between the arms in hematological toxicity. The leukopenia was more severe in the combination arm while the thrombocytopenia was more severe in the melphalan arm; however neither difference was significant (p = 0.5). In the melphalan arm, one patient experienced a toxic death while neutropenic and two had fever and neutropenia. In the melphalan plus misonidazole arm, three patients experienced anaphylaxis probably secondary to misonidazole.

DISCUSSION

This study reports the first randomized trial of a chemosensitizer, and demonstrates a statistically significant improvement in response rate among patients with non-small cell lung cancer treated with melphalan combined with misonidazole when compared with the same dose of melphalan alone. Although the low response rate observed in this study does not suggest a major therapeutic benefit for the patients treated, it does suggest that the cytotoxicity of a relatively inactive alkylating agent can be enhanced by agents such as misonidazole which lack cytotoxicity when administered alone. The 0% response rate achieved for melphalan alone in this trial is lower than that achieved with some other alkylating agents used as single agents (19). However, the 0% response rate with melphalan in the current study is only slightly lower than the 7% overall rate of response in non-small cell lung cancer reported in early single-agent melphalan studies. Furthermore, the early studies of single agents frequently did not utilize the more rigorous contemporary criteria for objective response and thus the historical 7% response rate may be an underestimate. This study confirms the laboratory observation that a chemotherapeutic agent need not have a high degree of antitumor activity by itself in order to be potentiated by a chemosensitizing agent (24).

That the hypoxic radiosensitizers were also found to possess the property of chemosensitization was recently described (4). The precise mechanism of chemosensitization is not known, but the presence of tumor hypoxia appears to be necessary for sensitization to occur (5–9). Reduction products of misonidazole are formed under exposure to hypoxia and are felt to be responsible for the chemosensitization. The requirement for hypoxia suggests that this approach would not be useful for micrometastatic disease unless hypoxia was present (33).

Following the administration of melphalan, a number of hours is required for DNA cross-linking to occur (34, 35). Unlike the situation with radiosensitization, in which the drug must be present at the time of irradiation, chemosensitization can occur when the sensitizer is given before or after the chemotherapeutic agent. Although the precise site of action of chemosensitization is unknown, data from Taylor et al. (34, 36) and Mulcahy (37) indicate that there is no interference with monoadduct formation. Rather, the enhancement occurs after the monoadduct formation and may involve inhibition of the repair of the monoadduct or alteration of the repair of the DNA-DNA cross-link.

In the laboratory, a wide range of 2-nitroimidazole compounds function as chemosensitizers for the alkylating agents and nitrosoureas, the two classes of drugs that are sensitized (8, 13, 37–44). However, not all alkylating agents and nitrosoureas are potentiated to the same extent by a specific 2-nitroimidazole. For example, CCNU is better enhanced by the very...
lipophilic sensitizer benznidazole than by misonidazole (39, 44) while for cyclophosphamide misonidazole is more effective than benznidazole (40). This may be due, in part, to some difference in the mechanism of sensitization of the nitrosoureas compared to other alkylating agents (41, 43).

Early studies in mice using a single dose of misonidazole demonstrated that it could alter the pharmacokinetics of melphalan, but experiments using a multidose misonidazole regimen designed to duplicate human plasma pharmacokinetics demonstrated that pharmacokinetic interaction between the agents was not occurring, and that chemosensitization occurred in the absence of altered plasma pharmacokinetics (14–16). Our data suggest that the pharmacokinetic profile of melphalan was altered minimally by misonidazole but the difference we observed was not statistically significant. The impact of this alteration cannot be assessed with certainty, although there was no enhancement of hematological toxicity in the misonidazole treated patients. This result indicates the importance of studying the pharmacokinetic parameters in randomized trials such as this.

There have been few trials studying the efficacy of chemosensitization. In a nonrandomized Phase II trial using CCNU with benznidazole for patients with metastatic melanoma, four partial responses were observed in 16 patients, providing equivocal evidence for chemosensitization (45). Glover et al. observed only one response in 30 patients treated with cyclophosphamide plus misonidazole for metastatic renal cancer (46).

The optimal dosage and scheduling of chemosensitizers remains to be established. It is not known whether the specific concentration of sensitizer is important (47) or whether a lower concentration for a more prolonged period would be superior (48). The misonidazole plasma concentration in this study was 100 ng/ml or greater for four of the five patients studied. This value is above the threshold suggested by Randawa et al. (47). Another sensitizer, such as SR 2508, could be used at a higher value is above the threshold suggested by Randawa et al. (47). or whether a lower concentration of sensitizer is important (47) or whether a lower concentration for a more prolonged period would be superior (48).

In conclusion, in this randomized Phase II trial misonidazole enhanced the clinical activity of the alkylating agent melphalan without enhancing normal tissue toxicity. This observation has implications for future clinical trials as alkylating agents are useful chemotherapeutic agents for many tumor types. With the demonstration of a dose-response relationship for alkylating agents it is not known whether the specific dose of chemotherapeutic agent plays a role in an interaction of competing mechanisms. Br. J. Cancer, 50: 305–316, 1984.


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