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Difluoromethylornithine and Leukocyte Interferon: A Phase I Study in Cancer Patients*

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Abstract—*Difluoromethylornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase, and human leukocyte interferon (IFN-α) have synergistic anti-tumor activities* in vivo *in* B 16 *melanoma and* in vitro *against several human cancer cell lines. We have, therefore, carried out a phase I combination study with DFMO plus alpha interferon in the following manner: DFMO was maintained at a steady dose for the first four levels,* 1.5 g/m^2 *every* 6 hr. *IFN-α was given in* 100% *increments ranging from* 0.4×10^6U/m^2 *to* 3.2×10^6U/m^2 *i.m. daily. At the fifth dose level both IFN-α and DFMO were raised by* 100 *and* 50% *respectively. From levels one through four the combination was well tolerated with no dose interruptions required because of G.I. toxicity or myelosuppression. However, at dose level* 5, *one-third of the patients required dose cessation and decrease due to nausea, vomiting and diarrhea. We conclude that for phase II studies the maximal tolerated dose is* 3.2 million units *of IFN-α*/m^2 *and* 1.5 g/m^2 *of DFMO every* 6 hr.

Of 12 *patients with metastatic melanoma,* 2 *had partial remissions lasting* 58+ *and* 36+ weeks. *Two additional patients had minor responses lasting* 29 *and* 32+ weeks. *Minor responses were observed in a patient with colon carcinoma and a patient with renal carcinoma.*

The clinical activity of the combination is currently being pursued in a phase II study among patients with metastatic malignant melanoma.

INTRODUCTION

DIFLUOROMETHYLORNITHINE (DFMO), an irreversible inhibitor of ornithine decarboxylase, blocks conversion of ornithine to putrescine, which is the first step in polyamine biosynthesis [1]. Polyamines are essential in cellular growth [2,3], and high concentrations of polyamines were observed in some malignancies [4]. The antiproliferative activities of DFMO have been demonstrated in several tumor cell lines [5] and animal models [6,7]. Human leukocyte interferon (IFN-α) has shown activity against several human solid tumors [8,9] and human hematopoietic malignancies [10,11]. Following the observations that the combination of DFMO and IFN-α have either synergistic or additive antitumor activity *in vitro* and *in vivo* in animal tumors [12–14], we initiated a phase I study to evaluate the tolerance, toxicity, and clinical activity of the combination.

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PATIENTS AND METHODS

Patient population

The patient population in this study included 25 men and women between the ages of 21 and 70 with measurable cancers. The types of cancers and previous treatments with chemotherapy are shown in Table 1. Twenty-one of the patients had 100% performance status on Karnofsky's scale. The inclusion criteria were life expectancy of at least 3 months, preserved kidney functions (creatinine < 2 mg%), preserved liver functions (bilirubin < 1.5 g%, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) < 150 U/ml), and adequate function of the bone marrow (granulocytes > $1.5 \times 10^3/\mu$l, platelets > $100 \times 10^3/\mu$l). A written informed consent was obtained according to institutional policies.

Prior to initiation of treatment, all patients had complete history and physical examinations, complete blood counts (CBC) and white blood cell differential counts, hepatic and renal function tests, serum electrolytes, urinalysis, electrocardiogram and audiogram. Measurable lesions were documented by physical examination and with

Table 1. Patient population treated with DFMO and IFN-α

Tumor type	Number of patients	Number with prior chemotherapy
Malignant melanoma	12	8
Colon cancer	4	3
Renal cell carcinoma	4	3
Chronic myelogenous leukemia		
Benign phase	2	1
Blast crisis	1	—
Large cell carcinoma of lung	1	1
Breast cancer	1	1
Total	25	17

appropriate radiological tests and tumor markers in patients with solid tumors. CBC and bone marrow aspiration and biopsy analyses were used to document disease in patients with leukemia.

Evaluation during treatment consisted of weekly physical examinations. Hemograms were performed every other day in the first 14 days and then weekly. Serum chemistry analysis and serum electrolyte evaluations were done twice weekly during the first 14 days and then weekly. Audiometry, electrocardiogram and evaluation of measurable disease were done at monthly intervals.

Drugs

DFMO was supplied by Merrell Dow Pharmaceuticals (Cincinnati, Ohio) in aqueous solution at a concentration of 3 g/15 ml. It was administered orally every 6 hours.

Human leukocyte interferon (IFN-α) was prepared as previously described [15], and was obtained from the State Serum Institute, Helsinki, Finland. It had a specific activity of $1–3 \times 10^6$ U/mg of protein and was administered by a single intramuscular (i.m.) injection each day.

Treatment schedule

Doses of the IFN-α and DFMO were administered at 5 treatment levels (Table 2). Each course consisted of 14 days on treatment and 3 days off treatment. The treatment dose was escalated in the individual patient if treatment toxicity was mild (grade 1). In addition 3 new (untreated) patients were studied at each dose level beyond level 1. In the first 4 courses [1–4] the DFMO dose was fixed at 1.5 g/m^2 every 6 hr. This dose of DFMO was based on preliminary European studies in both humans and animals showing mild toxicity and polyamine suppression at this dose level. (Data on file, Merrell-Dow.) The IFN-α dose was started at 0.4×10^6 U/m^2 and escalated by 100% from course to course in the first 4 courses. In the fifth course the doses of DFMO were increased to 2.25 g/m^2 every 6 hr while that of IFN-α was escalated to 6.4×10^6 U/m^2.

Toxicity criteria

The toxicity grades were defined as follows:

1. Gastrointestinal (GI) toxicities: Grade 1 was defined as nausea and vomiting 1 time daily and/or less than 3 loose stools daily; grade 2 was defined as less than 6 episodes of vomiting daily controlled by medication, and/or 3–6 loose stools daily; and grade 3 was defined as more than 6 episodes of vomiting and/or more than 6 loose stools daily.
2. Fatigue: Grade 1 was defined as mild fatigue that did not inferfere with common daily activity; grade 2 was defined as the need for bed rest of 3–6 hrs daily. Grade 3 was defined as the inability to perform common daily activities and fatigue requiring more than 6 hours of bed rest during the day.

Response criteria

Response to treatment was designated in the following manner: Complete remission (CR) was defined as the disappearance of all measurable lesions for at least 1 month. Partial remission (PR) was defined as a greater than 50% decrease in size of the sum of the products of measurable lesions for at least 1 month. Minor response was defined as a

Table 2. Treatment doses and clinical side effects of DFMO + IFN-α

	Dose levels		GI symptoms Grades 2 & 3	Fatigue Grades 2 & 3	Anemia Hgb < 10G/dcl	Thrombocytopenia, platelet count < 100 × 10^3/μl
	IFN-α × 10^6 U/m^2	DFMO g/m^2/q 6 hr				
1.	0.4	1.5	1/6	1/6	0/6	1/6
2.	0.8	1.5	2/8	2/8	0/8	0/8
3.	1.6	1.5	2/11	4/11	1/11	0/11
4.	3.2	1.5	3/14	4/14	2/14	0/14
5.	6.4	2.25	7/15	5/15	1/15	3/15
	Total		15/54	16/54	4/54	4/54

less than 50% decrease in size of the sum of the products of measurable lesions for at least 1 month. Appearance of new lesions or enlargement of existing lesions represented progressive disease. In the case of chronic myelogenous leukemia, in benign phase or blastic crisis, > 50% decrease in WBC and/or platelet counts (in case of thrombocytosis), or blast counts, but without normalization of peripheral blood count and morphology, were considered minor responses.

RESULTS

The most common toxicities that were associated with the study are shown in Table 2.

GI toxicities consisting of diarrhea, nausea and vomiting were the most significant, while weight loss, lack of appetite and abdominal discomfort were seen occasionally.

The incidence of grade 2 and 3 GI toxicities was consistently low in the first 4 dose levels but increased to occur in nearly 50% of the patients treated with level 5. These toxicities were of acute nature and were rapidly reversible during the interval between treatment courses. Four of 15 patient courses on level 5 were stopped because of GI toxicity, and then resumed at level 3, following recovery from the toxicity. Fatigue of grades 2 and 3 was seen in about 25% of the patients. Its incidence did not change, however, between treatment levels 2 and 5.

The hematologic toxicities shown in Table 2 were generally mild. However, thrombocytopenia with platelet counts of less than $100 \times 10^3/\mu l$ occurred in 4 patients, 3 of whom were treated at level 5. Among these 4 patients, the platelets ranged from $32 \times 10^3/\mu l$ to $86 \times 10^3/\mu l$, while platelet recovery to levels above $100 \times 10^3/\mu l$ occurred within 3–5 days after the end of the course. A fall in hemoglobin levels below 10 gm/dcl was uncommon and occurred in 4 of the 54 treatment courses. A progressive decline in the granulocyte counts from a mean of $1.7 \times 10^3/\mu l$ to a mean of $1.3 \times 10^3/\mu l$ was seen with dose changes from levels 1 to 5. It was, however, rapidly reversible within the 3 days of treatment interruption between courses; granulocytopenia was not associated with any case of severe infection.

Transient neurogenic hypoacusia developed in 18 of the 25 patients after a mean time of 6.4 weeks (range 2–10 weeks). Of the 18 patients, decreased hearing acuity was clinically detected in 9, while the remaining 9 patients had changes detected only by audiogram. The mean total dose of DFMO given before the development of toxicity was 587 g (range 142–1101 g). Following DFMO discontinuation, complete recovery of hearing was noted after a mean period of 8.7 weeks (range 3–12 weeks).

Less common toxicities included transient hypomagnesemia (2 patients), and marked but transient elevation of hepatic enzymes (SGPT ≥ 350 mU/ml) in 2 patients that resolved with either dose reduction or treatment interruption.

The tumor responses are presented in Table 3. Patients with metastatic malignant melanoma comprised the largest group. Of the 12 melanoma patients, 2 had partial responses sustained for 58+ and 36+ weeks and 2 had minor responses. Minor responses were also seen in a patient with colon cancer and a patient with renal cancer.

DISCUSSION

IFN-α, a naturally occurring protein with antitumor activities, was shown to be synergistic with DFMO, a suicide inhibitor of the enzyme ornithine decarboxylase which depletes polyamines [12–14]. Previous studies with DFMO alone showed polyamine suppression at a dose of 6 g/m ^{2}/daily. Following a DFMO phase I study in cancer pa-

Table 3. Clinical responses on DFMO and IFN-α

Disease	Patients studied	Complete remission	Partial remission	Minor response	Progressive disease
Malignant melanoma	12	0	2*	2	8
Renal cell carcinoma	4	0	0	1	3
Colon cancer	4	0	0	1	3
Chronic myelogenous leukemia	3	0	0	3	0
Others	2	0	0	0	2
Total	25	0	2	7	16

* Lasting for 36+ and 58+ weeks.

tients, Abeloff recommended a dose of DFMO ranging from 2 to 3 g/m^2 every 6 hr when given alone [16]. We elected, therefore, to combine a fixed dose of DFMO of 1.5 g/m^2 every 6 hr with escalating doses of IFN-α for the first 4 dose levels. In the fifth dose level, we escalated the doses of both DFMO and IFN-α.

The first 4 dose levels were tolerated well; however, the increase of the DFMO dose to 2.25 g/m^2 every 6 hr and the IFN-α to 6.4 × 10^6 U/m^2 daily was associated with an increase in grades 2 and 3 gastrointestinal toxicities which occurred in about 50% of the patients and led to treatment interruption in 4 patients. This extent of GI toxicities was not seen when DFMO was given alone even at doses of 3 g/m^2 every 6 hr [16]. It is possible, therefore, that the addition of IFN-α had aggravated the gastrointestinal toxicities.

Thrombocytopenia represented the most significant hematologic toxicity, but toxicity was less frequent and of shorter duration than the one seen with DFMO alone, perhaps due to the larger number of previously untreated patients in our study and their generally better performance status.

Hearing loss was observed in 72% of our patients and was reversible after discontinuation of DFMO. Future studies employing oral DFMO should be designed in a way that will allow recovery from this toxicity.

On the basis of the observed toxicities, we recommend the use of oral DFMO dose of 1.5 g/m^2 every 6 hr combined with 3.2 × 10^6 U/m^2 of IFN-α daily. This was further confirmed in studying 7 additional patients with DFMO of 2–2.25 g/m^2 every 6 hr and 1.5 × 10^6 U/m^2 of IFNα, when grade 3 GI toxicity developed in 4 of the 7 patients; thus, any increase in the DFMO dose beyond 1.5 g/m^2 every 6 hr was associated with unacceptable toxicity. Therefore, treatment should be administered at the aforementioned recommended doses until hearing changes upon audiogram, at which time DFMO administration should be interrupted until complete hearing recovery. Then DFMO should be resumed.

Clinical activity of the combination of DFMO and IFN-α was observed in melanoma patients resulting in PR in 2 of 12 patients. Treatment with DFMO alone at doses of 1.5 g/m^2 every 6 hr did not result in remissions among melanoma patients (Dr. Meyskens' personal communication), while only 1 PR was observed in 44 melanoma patients treated with IFN-α at doses of 3–9 × 10^6 U/i.m. daily [17]. However, detailed phase II studies and controlled studies will be required to evaluate the possible therapeutic advantage of the combination.

The anti-neoplastic effect of DFMO may result from inhibiting one or several cellular activities which require polyamines [19–23]. More than one mechanism may also be associated with the antiproliferative activity of IFN-α [24–28], and the combination of both DFMO and IFN-α may inhibit more than one process associated with neoplastic proliferation. One possible target for the synergistic activity may be the enzyme ornithine decarboxylase (ODC) which is the rate limiting enzyme in the polyamine biosynthesis and was shown to be inhibited by DFMO [1] and interferon as well [26].

DFMO also enhances, *in vitro*, the antitumor activity of several cytotoxic agents [29–31] so that, in addition to the evaluation of the therapeutic potential of IFN-α and DFMO combination in several tumors, their interaction with other treatment modalities should also be studied.

DFMO was recently administered intravenously, in systemic protozoal infections (Merrell-Dow unpublished data), and in acute leukemia patients (Dr. A. M. Maddox, unpublished data). The much higher doses utilized in these studies were associated with reduced GI toxicities. We have started, therefore, to use the above combination with DFMO administered intravenously, and thus far we have noticed a marked reduction in GI toxicities. Continuous administration of doses up to 12 g/m^2 daily was not associated with GI symptoms but was limited by the development of thrombocytopenia (M. Talpaz, unpublished data). This approach may improve the patient's tolerance to the combination and it will be evaluated in further studies.

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