Title
A phase II study of alpha-difluoromethylornithine (DFMO) for the treatment of metastatic melanoma.

Journal
Investigational new drugs, 4(3)

ISSN
0167-6997

Authors
Meyskens, F L
Kingsley, E M
Glattke, T
et al.

Publication Date
1986

Peer reviewed

Investigational New Drugs 4: 257-262, 1986

A phase II study of α-difluoromethylornithine (DFMO) for the treatment of metastatic melanoma

Frank L. Meyskens, Edwin M. Kingsley, Theodore Glattke, Lois Loescher & Ann Booth
Departments of Internal Medicine, Speech and Hearing Science, Cancer Center Division, University of Arizona, Tucson, AZ 85724, USA

Key words: difluoromethylornithine, melanoma, polyamine, ornithine decarboxylase

Abstract

Difluoromethylornithine (DFMO) is an irreversible enzyme-activated inhibitor of ornithine decarboxylase, a key enzyme in polyamine synthesis. We have screened for potential anti-cancer activity of DFMO using a clonogenic assay, which suggested that melanoma might have sensitivity to this agent. Accordingly, we have performed a phase II trial of DFMO (2 g/m² po q 8 h) in 24 patients, 21 of whom were evaluable for response. One patient achieved a complete response of a large subcutaneous mass for 11 months. Although stabilization is frequently difficult to measure, seven patients appeared to stabilize previously active disease, with a median duration of response of eight weeks. Toxicity was significant and DFMO was discontinued in five patients due to side effects – hearing loss alone in four and hearing loss associated with thrombocytopenia in the fifth patient. Hearing changes occurred in ten patients. Other side effects were mild. These data indicate that DFMO as a single agent may be an effective therapy for melanoma. A phase II trial of DFMO in previously untreated patients using a different schedule to decrease hearing loss is warranted. Additionally, several in vitro and animal models suggest that DFMO plus interferon are synergistic, and this combination might be used for a clinical trial as well.

Introduction

The resistance of metastatic melanoma to standard chemotherapy has spurred the investigation of new cytotoxic agents over the past decade. Unfortunately, little progress has been made and DTIC remains the single most active cytotoxic drug (1). Only 20% response rates have been achieved, and the impact on overall survival has been negligible. We and others have therefore begun to study alternative approaches, including biological response modifiers (such as the interferons) and biochemical modulators.

DFMO is an enzyme-activated irreversible inhibitor of ornithine decarboxylase (2, 3) a key enzyme in the synthesis of two polyamines, putrescine and spermidine, both of which are intimately associated with cellular proliferation and growth (4). The antiproliferative activity of DFMO has been demonstrated both in cultured cells (5) and in tumor-bearing animals (6, 7). Of particular interest is the apparent potentiating effect of DFMO on more conventional cytotoxic agents, such as the nitrosoureas, in the treatment of tumor-bearing animals (7, 8). In addition, a recent report documented a synergistic antiproliferative effect of DFMO in vitro when combined with either recombinant leukocyte type A human interferon or doxorubicin (9).

There is very little information concerning the

Address for offprints: Frank L. Meyskens, Jr., MD., Arizona Cancer Center, University Medical Center, Room 6335, University of Arizona, Tucson, AZ 85724, USA.

activity of DFMO in human malignancy. Siimes et al. has treated five patients with refractory lymphoblastic or myeloblastic leukemia using a combination of DFMO and a second polyamine antimetabolite, methylglyoxal bis(guanylhydrazone) (MGBG), with only minimal toxicity (10). The synergism of these two agents appeared to be due to increased leukemic cell uptake of MGBG when preceeded by treatment with DFMO. Abeloff et al. have recently reported a phase I trial of DFMO, and thrombocytopenia was identified as the limiting serious side effect (11).

We have utilized a clonogenic assay to screen for the activity of new anticancer agents for some time (12). We and others have demonstrated that the assay has utility in selecting for clinical response to cytotoxic agents, as well as to biologic response modifiers such as the retinoids and interferon (13, 14). Based on activity in the in vitro screen, we have selected metastatic melanoma for a phase II study and the results of this trial are reported here.

Materials and methods

Measurement of effect of DFMO on human tumor colonies in soft agar

We have published in detail on the use of colony formation as an indicator for sensitivity to drugs and other compounds (12). Briefly, tumor specimens were obtained aseptically, processed into single cell suspensions, and plated in the upper well of a bilayer agar culture. DFMO was added directly to the upper layer. Cultures were incubated for 10 to 14 days and colonies scored using an automatic optical scanner (FAS II). This assay may be useful for screening of new anti-cancer drugs; criteria for in vitro sensitivity have been presented elsewhere (12–14).

Patient eligibility and selection

Between March 1983 and March 1985, 24 patients with histologically confirmed metastatic melanoma were entered into study. Written informed consent was obtained for all patients based on a protocol approved by the University of Arizona Institutional Review Board. Demographic data for the 21 evaluable patients is shown in Table 1. Median age was 62 years with a range of 26 to 79. 52% had a Karnofsky performance status (PS) of 90–100%, 29% had a 70–89% PS, and 19% had a 60–69% PS. Predominant sites of metastatic disease were gastrointestinal and/or brain in nine patients, lung and/or bone in 13, skin and/or nodes in 20 and other in one. Seventeen patients had received prior chemotherapy. No concomitant radiation treatment or chemotherapy within 21 days (42 days for nitrosoureas) of beginning DFMO was allowed. Baseline history, physical examination, liver and kidney chemistries, complete blood counts, chest radiograph, urinalysis and audiogram were obtained in all patients. Adequate bone marrow function (WBC $\geq$ 4000/cc, platelets $\geq$ 100,000/cc), hepatic function (bilirubin $\leq$ 2.0 mg%) and renal function (creatinine $\leq$ 1.5 mg%) were documented in all patients. A life expectency of at least 12 weeks and objectively evaluable disease were required. Patients were followed every 2 weeks with blood tests and every 4 weeks with physical examination, blood tests, urinalysis and audiogram, as well as tumor measurements.

Treatment plan

A starting oral dose of DFMO 2 g/m^2 every 8 h was used in all patients but two, both of whom were begun at 3 g/m^2. The dose was increased (to a maximum of 4 g/m^2) if the disease was stable and toxicity was tolerable. Treatment was continued until disease progression or intolerable toxicity not ameliorated by reducing the dose of DFMO.

Tumor response criteria

Response was classified as either a complete response (CR), a partial response (PR), stable disease (SD), or as progressive disease (PD). CR was defined as complete disappearance of all clinical, radiographic, and biochemical evidence of

Table 1. Characteristics of 21 patients with metastatic melanoma evaluable for response to DFMO.

Median age (range)	62 years (26–79)
Male:female ratio	9:12
Performance status (Karnofsky)	
90–100%	11
70–89%	6
50–69%	4
Prior treatment	17
Sites of metastatic involvement	
Skin	12 (23%)
Lung	11 (21%)
Lymph nodes	8 (16%)
Liver	5 (10%)
Brain	3 (6%)
Bone	2 (4%)
Bowel	1 (2%)
Bladder	1 (2%)

disease for at least 4 weeks. PR was defined as a 50% decrease in the sum of the products of the longest diameter and its perpendicular for all measurable sites, lasting for at least 4 weeks. SD was defined as a less than 50% decrease but not greater than a 25% increase of measurable disease maintained for at least 4 weeks. All other patients were classified as having PD.

Results

Response of tumor colony forming units to DFMO

We measured the effect of continuous exposure to DFMO on tumor colony forming units (TCFU) from 106 solid tumors (Table 2). These results suggested that melanoma might be a good tumor to target for a phase II study inasmuch as TCFU were reduced to less than 50% in 32% of the cases and below 30% in 15% of cases. Based on prior experience with the assay we predicted a clinical response rate of 10–15%.

Clinical effect of DFMO

Three patients were considered nonevaluable for purposes of this study. Patient #9 was lost to follow-up within 1 month of beginning treatment. Patients #10 and #13 refused any DFMO after signing consent forms. Of the remaining 21 patients, one achieved a CR (5%), seven exhibited SD (33%), and the remaining 13 patients experienced PD (62%). Previously treated patients appeared to respond less frequently to DFMO (3/16 with SD)

Table 2. Effect of continuous exposure to DFMO on human tumor colony formation in vitro[a]

Tumor type	No. of patients tested	No. of patients[b]		
		Sensitive (< 30%)	Intermediate (30–50%)	Resistant (>50%)
Melanoma	47	7 (11)	8 (10)	32
Ovary	19	0 (2)	2 (2)	17
Lung	10	0	1 (1)	9
Breast	7	0	1 (2)	6
Miscellaneous solid tumors[c]	24	0	2 (4)	22
Total percentage	107	7 (13) 6.5 (12)	14 (19) 13 (18)	86 70

[a] The effect of continuous exposure to 0.50 and 5.0 mM on TCFU was measured. Number in parenthesis is number of patients with reduction of TCFU after continual exposure to DFMO at a concentration of 5.0 mM.

[b] Reduction of colonies below 30% was designated as sensitive and between 30 and 50% as intermediate as described in Materials and Methods.

[c] Miscellaneous solid tumors include the following diagnoses (and number): sarcoma (3), corpus uteri (3), colonecta (3), kidney (3), unknown primary (4), brain (2), cervix uteri (3), pancreas (2), thyroid (1).

Table 3. Treatment results in 21 evaluable patients with metastatic melanoma treated with DFMO according to metastatic site.

Metastatic site	Number of responses (percentage)		
	CR	SD	PD
Skin	1	2	9
Lung		4	7
Lymph nodes		2	6
Liver		3	2
Brain			3
Bowel			1
Bone		1	1
Bladder		1	
Total (sites)	1	13	29
Total (patients)	1 (5%)	7 (33%)	13 (62%)

compared with patients not previously treated (1 CR, 4 SD in five patients). All metastatic sites except brain and bowel exhibited response to DFMO (Table 3). There was no correlation between disease response to DFMO and performance status or gender (data not shown).

Patient #11 who achieved a CR, was begun on DFMO for a painful left flank mass measuring 16 × 24½ cm, which had been previously biopsied and histologically confirmed as melanoma. Two weeks later there was marked pain reduction and the mass measured 7 × 1½ cm. After 2 months of treatment (3/20/84) the pain and mass had completely resolved. The patient discontinued DFMO after 5 months and remained in a CR when evaluated 1 month later. Five months after drug discontinuation, the patient relapsed with a large mass in the right axilla. No left flank mass was evident. The median duration of response in patients achieving SD was 8 weeks (range 4–12 weeks).

Toxicity of DFMO

The toxicity of DFMO at doses administered in this study was significant. The drug was discontinued in five patients due to side effects – hearing loss alone in four and hearing loss associated with thrombocytopenia in the fifth patient.

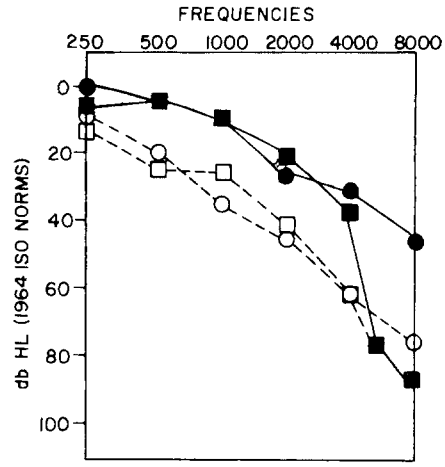

Fig. 1. Pre- and post-therapy audiogram of a patient receiving DFMO. Pre-solid, post-open: ■, □ right ear, ●, ○ left ear.

Hearing changes occurred in ten patients (48%), although this was documented by pure tone audiometry in only six. In three patients the hearing loss was characterized audiographically as bilateral, sensoneural, primarily high frequency, with a median decibel loss of 25–30. A representative pretherapy and posttherapy audiogram for one of these patients is shown in Fig. 1. All experienced audiographic-documented hearing recovery within 1–3 months following cessation of DFMO. In three patients, unilateral hearing loss of 10–15 decibels was evident and was fluctuating (Menière's-type) in two. There was no apparent association between total drug dose and degree of hearing loss (data not shown).

There was only one severe episode of thrombocytopenia (40,000) and the platelet fall ceased within one day after stopping DFMO and returned to normal within 10 days.

Other DFMO side effects were generally mild (grade 1–2) and did not result in any drug cessation (Table 4). There were a total of 31 episodes of grade 1 toxicity (50% of all toxic events), 23 episodes of grade 2 toxicity (37%) and nine grade 3 toxic events (13%). Gastrointestinal (GI) side effects accounted for the majority of the toxic episodes (67%) with neurologic side effects accounting for 8% and others 25%.

Table 4. Toxicity of DFMO in 21 patients.

Toxicity	Grade	No. of episodes			
		1	2	3	4
GI					
Anorexia		3	2	2	
Dysphagia			2		
Stomatitis		2		1	
Nausea/vomiting		8	6	1	
Abdominal pain		1			
Diarrhea		1	5	3	
Flatulence		5			
Neurologic					
Hearing loss		10			
Tinnitus		1			
Dizziness		1	1	1	
Depression			1		
Hematologic					
Thrombocytopenia				1	
Other					
Fatigue		7	4		
Rash		1	1		
Arthralgias		1			
Alopecia			1		
Total		31	23	9	0

Discussion

Based on this phase II study, DFMO demonstrated limited activity in patients with metastatic melanoma. One patient experienced a complete response. Seven patients experienced transient stabilization of their disease and the majority (13) experienced disease progression. Outside the confines of a prospective randomized study, the significance of apparent stability of disease must be interpreted cautiously inasmuch as melanoma can be a notoriously unpredictable tumor.

Toxicity to DFMO was significant as reflected by drug cessation in five patients (24%) due to intolerable side effects. Hearing changes were the most significant toxicity, occurring in almost 50% of evaluable patients and accounting for discontinuation of drug in all five patients. There was no apparent association between total DFMO dose and degree of hearing loss. All affected patients experienced clinical recovery of hearing deficits within 1 to 2 months of discontinuing the drug.

GI toxicity was the most common side effect, with nausea and vomiting occurring in 67% of evaluable patients. The majority of the toxic episodes were grade 1 or 2, however, and in no case was DFMO discontinued as a result of this side effect. 50% of the patients experienced fatigue while taking DFMO, but it was grade 1 or 2 in all cases. There was only one serious hematologic toxicity (thrombocytopenia) which, combined with hearing loss, resulted in discontinuation of DFMO in one patient. This is in contrast to the study of Abeloff et al. (11), in which 11 of 16 patients who received intravenous DFMO and who had received prior therapy experienced dose limiting thrombocytopenia. No permanent sequelae as a result of DFMO toxicity, including hearing loss, could be documented in any patient.

Additional studies of DFMO-containing regimens should be undertaken, both in vitro and in vivo. Particularly close attention should be paid to monitoring for both hearing and hematopoietic changes. Because of the inordinate amount of ototoxicity demonstrated in this study, however, we recommend that future studies using DFMO utilize a lower starting dose or an interrupted schedule, the latter approach supported by the recent results of Natale et al. (15). A phase II study in previously untreated patients with melanoma is warranted. Several in vitro animal models suggest that DFMO and interferon are synergistic, and this combination might be used for a clinical trial also.

Acknowledgments

We thank R. Goldman for excellent secretarial assistance.

Supported in part by grants from the National Cancer Institute (CA17094, CA27502, CA23074) and Merrell Dow Research Institute (Cincinnati, Ohio).

References

1. Comis RL: DTIC (NSC-45388) in malignant melanoma: a perspective. Cancer Treat Rep 60:165–176, 1976
2. Mamont PS, Duchesne M, Juder-Ohlenbusch A, Grove J: Effects of ornithine decarboxylase inhibitors on cultured cells. In: Seller N, Jung MJ, Koch-Weser J (eds): Enzyme-activated irreversible inhibitors, Vol. 3. Elsevier/North-Holland Biomedical Press, Amsterdam, 1978, pp 43–53
3. Metcalf BW, Bey P, Danzin C, Jung MJ, Casara P, Vevert JP: Catalytic irreversible inhibition of mammalian ornithine decarboxylase (EC 4.1.1.17) by substrate and product analogues. J Am Chem Soc 100:2551–2553, 1978
4. Pegg AE, McCann PP: Polyamine metabolism and function. Am J Physiol 243 (Cell Physiol 12):C212–C221, 1982
5. Mamont PS, Duchesne MC, Grove J, Bey J: Antiproliferative properties of D, L-α-difluoromethylornithine in cultured cells. A consequence of the irreversible inhibition of ornithine decarboxylase. Biochem Biophys Res Commun 81:58–66, 1978
6. Prakash NJ, Schechter PJ, Grove J, Koch-Weser J: Effect of α-difluoromethylornithine, an enzyme-activated irreversible inhibitor of ornithine decarboxylase on L1210 leukemia in mice. Cancer Res 38:3054–3062, 1978
7. Marton LJ, Levin VA, Hervatin SJ, Koch-Weser J, McCann PP, Sjoerdsma A: Potentiation of the antitumor therapeutic effects of 1,3-bis(2-chloroethyl)-nitrosourea by α-difluoro-methylornithine, an ornithine decarboxylase inhibitor. Cancer Res 41:4426–4431, 1981
8. Sano Y, Deen DF, Oredsson SM, Marton LJ: Effects of α-difluoromethyl-ornithine on the growth of 9L rat brain tumor multicellular spheroids and their response to 1,3-bis(2-chlorethyl)-1-nitrosourea. Cancer Res 144:577–581, 1984
8. Salmon SE, Hamburger AW, Soehnlen B, Durie BG, Alberts DS, Moon TE: Quantitation of differential sensitivity of human-tumor stem cells to anticancer drugs. N Engl J Med 298:1321–1327, 1978
9. Kovach JS, Svingen PA: Enhancement of the antiproliferative activity of human interferon by polyamine depletion. Cancer Treat Rep 69:97–103, 1985
10. Siimes M, Seppanen P, Alchonen-Hongisto L, Janne J: Synergistic action of two polyamine antimetabolites leads to a rapid therapeutic response in childhood leukemia. Inst J Cancer 28:567–570, 1981
11. Abeloff MD, Slavik M, Luk GD, Griffin CA, Hermann J, Blanc O, Sjoerdsma A, Baylin SB: Phase I trial and pharmacokinetic studies of α-difluoromethylornithine – an inhibitor of polyamine biosynthesis. J Clin Oncol 2:124–130, 1980
12. Salmon SE, Meyskens FL Jr, Alberts DS, Soehnlen B, Young L: New drugs in ovarian cancer and malignant melanoma in vitro phase II screening with the human tumor stem cell assay. Cancer Treat Rep 65:1–12, 1981
13. Meyskens FL Jr, Salmon SE: Inhibition of human melanoma colony formation by retinoids. Cancer Res 39:4055–4057, 1979
14. Salmon SE, Durie BGM, Young L, Lui RM, Trown PW, Stebbing N: Effects of cloned leukocyte interferons in the human tumor stem cell assay. J Clin Oncol 1:217–225, 1983
15. Natale RB, Meyer K, Forastiere A: Phase I trials of α-difluoromethyl-ornithine alone and in combination with methylglyoxal bis-guanylhydrazone. Proc ASCO 4:C-532, 1985